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THE ASSESSMENT OF THE CARCINOGENICITY OF DICOFOL (KELTHANE), DDT, DDE, AND DDD (TDE)

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THE ASSESSMENT OF THE CARCINOGENICITY OF DICOFOL (KELTHANE™), DDT, DDE, and DDD (TDE)

by

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16. ABSTRACT The carcinogenic activity of the pesticides dicofol and associated pesticide compounds DDT, DDE, and DDD are reviewed. All of these compounds exhibit carcinogenic activity in surrogate test animals. The largest literature base exists on DDT which indicates a positive carcinogenic activity in 13 separate biotests for cancer activity. The primary target organ was the liver, but in some tests lung tumors and leukemias were significantly increased. DDT is judged on the bases of these biotests, positive mutagenicity in vivo, two-stage chemical carcinogenesis tests, and the lack of relevant epidemiological tests to be probably carcinogenic to man. The EPA cancer classification is determined to be B2 based on a sufficient cancer response. Dicofol has only one test which is positive in the liver for carcinogenicity in B6C3F1 mice. DDE and DDD were also tested positive for carcinogenicity. Dicofol has a striking structural analogy to DDT, DDE, and DDD (all of which are categorized as B2). Dicofol is categorized in the B2 to C range and is considered to be at least possibly carcinogenic to man. Dicofol, DDT, DDE, DDD animal test data, when analyzed by the linearized multistage model for low-dose extrapolation, show similar cancer potencies: $q_1^* = 0.44, 0.34, 0.34, 0.25$, respectively, $(mg/kg/day)^{-1}$. Such similiarity in cancer potency values suggests that either a common carcinogenic metabolite is generated from these compounds, or each compound has intrinsic carcinogenic activity and need not be metabolized to any other compound in order to cause cancer.

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PREFACE

The Carcinogen Assessment Group (CAG) within the Office of Health and Environmental Assessment has prepared this dicofol (Kelthane™) cancer assessment at the request of and for the use of the Hazard Evaluation Division (HED), Office of Pesticide Programs, Office of Pesticides and Toxic Substances.

The scientific literature was reviewed on the carcinogenicity of dicofol as well as on the dicofol contaminants (and possible metabolites) DDT, DDE, and DDD. Those studies that exhibited adequate design, conduct, and reporting were employed to assess the carcinogenicity of dicofol and the related compounds DDT, DDE, and DDD. Furthermore, the upper bound cancer potency of these compounds was also determined in order to place an upper limit on the unit risk expected from dietary exposure to these compounds.

According to EPA's system for categorizing the evidence of carcinogenicity, dicofol has been assessed to be in the category range C to B2, based on one positive cancer study in mice and chemical inference from other structurally related compounds, such as DDT, DDE, DDD, and chlorobenzylate, which also show positive carcinogenic activity. The CAG has concluded that the weight of evidence for the carcinogenicity of dicofol is based on: no human evidence, one positive mouse study, one negative rat study, and on structural comparisons to other animal (and possibly human) carcinogens.

A comprehensive search of the scientific literature supporting this document is complete through January 1985.

The cancer category C to B2 range described and supported in this document was communicated to the Office of Pesticide Programs in a memorandum from Robert E. McGaughy (with attachments prepared by James W. Holder and Bernard H. Haberman), U.S. EPA, CAG, to John Melone, U.S. EPA, HED, June 20, 1985.

In the opinion of the CAG, the carcinogenicity of dicofol is best reflected by a range of C up to B2, which connotes that dicofol is at least possibly carcinogenic to humans and is likely to be intermediate between a possible human carcinogen (category C) and a probable human carcinogen (category B2). Further studies are indicated to delineate the extent to which dicofol is, or is not, a human carcinogen.

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1. SUMMARY AND CONCLUSIONS

A comprehensive literature search has been conducted by the Carcinogen Assessment Group (CAG) in order to determine the carcinogenic potential of dicofol and the associated compounds DDT, DDE, and DDD (also known as TDE). Dicofol was tested for carcinogenicity as the technical-grade material (reported by the Office of Pesticide Programs to be 85% to 90% active ingredient) which contains DDT, DDE, and DDD as impurities. In other studies technical-grade DDT, DDE, and DDD were each tested for carcinogenicity in 2-year bioassays.

In the case of DDT (the largest data base), 25 animal carcinogenicity studies are reviewed, including the following biotest species: mice, hamsters, rats, fish, dogs, and monkeys. Most of the positive tests that are reviewed (13 tests in all, including mice, rats, and fish) showed the liver to be the primary target site for DDT, although two studies showed only lung tumors and leukemias. The overall qualitative determination of the carcinogenic potential of DDT reveals adequate positive evidence in mice and limited positive evidence in rats and fish, while in contrast, adequate negative evidence is determined in hamsters and limited negative evidence in monkeys. The canine data are judged inadequate for determining the carcinogenic potential of DDT. The overall weight of evidence indicates that DDT has a more positive than negative carcinogenic character. The combined weight of evidence for the carcinogenicity of DDT from all of these studies is judged to be greater than one positive test species but not as great as two test species.

Additional qualitative evidence for the carcinogenicity of DDT in animals has been obtained from _in vivo_ two-stage initiation/promotion studies and from genotoxicity studies. In the initiation/promotion studies, DDT exhibited tumor

promotion activity in conjunction with a number of known carcinogens, including 2-acetylaminofluorene (2-AAF), 2-acetamidophenanthrene (AAP), and trans-4-acetylaminostilbene (trans-AAS). In genotoxicity studies, DDT showed negative effects in a number of studies and positive effects in others. The positive effects included point mutations, chromosome aberrations, increased sister chromatid exchanges, and direct interactions with DNA (all in eukaryotic cells). However, few of these genotoxicity studies have been replicated, and generally the positive effects were not measured in the same assays as the negative effects.

These additional observations, in the opinion of the CAG, elevate the weight of evidence for DDT to be equivalent with two positive test species. Epidemiologic evidence does not factor into the weight-of-evidence consideration for the carcinogenicity of DDT, since adequate epidemiologic data apparently do not exist at this time. Thus, according to the classification scheme of the International Agency for Research on Cancer (IARC), DDT is judged to belong in Group 2B. This classification is equivalent to EPA's Group B2 according to the Proposed Guidelines for Carcinogen Risk Assessment (U.S. EPA, 1984). This classification designates that there is a sufficient amount of animal carcinogenicity data to indicate the likelihood of cancer in man.

Dicofol was tested in both sexes of Osborne-Mendel rats and B6C3F1 mice in a National Cancer Institute (NCI) study reported in 1978. In this study, only male B6C3F1 mice responded with excess tumors (carcinomas of the liver). Normally, this singular set of observations would place the chemical in IARC Group 3 (or EPA's Group C), but since dicofol bears a close structural similarity to DDT, the EPA category is elevated. The likelihood that dicofol is a human carcinogen is considered to be in the range from possibly carcinogenic to humans to probably carcinogenic to humans. Therefore the weight of evidence for its carcinogenicity suggests a C to B2 range, using the 1984 Proposed

Guidelines for Carcinogen Risk Assessment. Further study is necessary to determine the extent to which dicofol may, or may not, be carcinogenic to humans.

The DDT metabolites DDE and DDD both demonstrated carcinogenic activity in animal biotests. Both the DDE and DDD metabolites retain a substantial structural similarity to DDT. Due to the carcinogenic activity of these metabolites, both DDE and DDD are judged to belong in IARC Group 2B (equivalent to EPA's Group B2).

The above qualitative considerations concerning the carcinogenicity studies of dicofol, DDT, DDE, and DDD indicate a sufficient level of carcinogenicity that it is deemed prudent, for purposes of risk estimation, to quantitatively estimate the expected cancer potency of these substances in humans. The actual extent to which these compounds are, in fact, carcinogenic to man remains to be established, since the appropriate epidemiologic studies are lacking. The lack of human epidemiologic data is unfortunate since DDT, DDE, and to a lesser extent DDD, are known to be persistent in the environment and in human tissues where DDT has been used. The persistence of dicofol has not been adequately reported.

In estimating the cancer potencies of dicofol, DDT, DDE, and DDD, the CAG has employed only adequately conducted and reported bioassays for carcinogenicity. The quantitative estimation of the upper-bound cancer potency showed that the oncogenic potential for DDT does not increase in multigeneration feeding experiments, but rather, remains approximately the same from generation to generation. However, the cancer potency estimates do vary from experiment to experiment, with only one DDT study being rejected as an outlier value. The remaining studies had values which were grouped closely enough so that an average estimate of cancer potency could be made. The average q_1^* values for all of the compounds reviewed are as follows:

Cancer potency	Dicofol	DDT	DDE	DDD
q_1^* $(mg/kg/day)^{-1}$	0.44	0.34	0.34	0.25

The q_1^* values for the upper-bound limit of cancer potency are judged by the CAG to be essentially the same for each of the above compounds. The closeness of q_1^* values among these compounds suggests either that all the compounds have a similar carcinogenic activity, or that they share a common metabolite or impurity which is the effector of the carcinogenic process.

Other studies that support the carcinogenicity of DDT to man (and presumably the other compounds by comparison) are two-stage initiation/promotion experiments and genotoxicity studies. DDT was found to operationally complete the subcarcinogenic doses of known rat carcinogens, thereby producing tumors in rats. Such activity is known to be characteristic of tumor-promoting compounds. The fact that DDT has been shown to interface with a number of rat carcinogens adequately demonstrates its tumor-promotion characteristics. Since tumor-promotion activity is also thought to be operative in man, this promotion activity in rats is seen as pointing to a similar activity in man.

Still other studies that supported the carcinogenicity of DDT and DDE to man (and presumably dicofol and DDD as well) are positive genotoxicity studies. In a number of genotoxicity studies in eukaryotic cells, DDT and DDE were found to be genotoxic. DDT did not cause genotoxicity in prokaryotic bacterial and fungal cells. In those studies that were positive for genotoxicity, DDT exhibited point mutations in V79 hamster cells, chromosome aberrations in cultured human lymphocytes, sister chromatid exchanges in V79 and CHO cells, and direct interactions with DNA in the presence of a cytosol activation system. These positive genotoxicity studies suggest that DDT may act as a tumor initiator. If DDT has both tumor-initiating and tumor-promoting characteristics, it can

be predicted that DDT should be able to act as a complete carcinogen. This is true for mice, in which DDT apparently does act as a complete carcinogen.

The CAG has determined, as a result of the above considerations, that dicofol, DDT, DDE, and DDD all have carcinogenic potential to man. On the basis of this determination, an upper-bound value for cancer potency of $q_1^* = 0.34\ (mg/kg/day)^{-1}$ has been estimated which can be employed in the risk management of these compounds. This cancer potency value is in the third quartile of the ranked potency values of compounds previously evaluated by the CAG.

2. INTRODUCTION

2.1. SCOPE OF REPORT CONCERNING DICOFOL AND RELATED COMPOUNDS DDT, DDE, AND DDD

The intent of this report is to assess the carcinogenicity of dicofol (Kelthane™), DDT, DDE, and DDD (also known as TDE). Evidence from human, animal, tumor-promotion, and genotoxicity studies is evaluated. These evaluations are combined into a weight-of-evidence determination of the carcinogenic potential of dicofol, DDT, DDE, and DDD. The weight of evidence indicates the likelihood that these substances are carcinogenic in humans, and therefore a quantitative cancer potency estimate is determined for each of these compounds.

The structure of dicofol, as well as the structures of the other compounds referred to in this report, are presented in Table 1. For purposes of comparison, Table 1 also includes some pesticides that are structurally related to dicofol.

The uptake, storage, metabolism, and metabolic interrelationships of DDT, DDE, and DDD have been discussed in detail elsewhere [World Health Organization (WHO), 1979; International Agency for Research on Cancer (IARC), 1974; U.S. Environmental Protection Agency (U.S. EPA), 1980a]. However, little is known at this time about the *in vitro* and *in vivo* metabolism of dicofol. The possibility exists that technical-grade dicofol (containing 85% to 90% active ingredient, plus the related compounds DDT, DDE, and DDD as contaminants) can metabolize to DDT-related compounds in the environment and *in vivo*. The metabolic interrelationships that could exist among dicofol and these DDT-related compounds are summarized in Figures 1 and 2.

Because of the close structural and possible metabolic relationships of DDT, DDE, and DDD to dicofol, the present report assesses the putative carcino-

TABLE 1. STRUCTURE OF DICOFOL AND OF p,p'-DDT AND ITS ANALOGUES OF THE FORM[a]

Name DDT and its major metabolite	Chemical name	R	R'	R"
dicofol[b] (Kelthane®)	4-chloro-α-(4-chlorophenyl)- (trichloromethyl)benzenemethanol	—Cl	—OH	$-CCl_3$
DDT[b]	1,1'-(2,2,2-trichloroethylidene)- bis[4-chlorobenzene]	—Cl	—H	$-CCl_3$
DDE[b,c]	1,1'-(2,2-dichloroethenylidene)- bis[4-chlorobenzene]	—Cl	None	$=CCl_2$
TDE(DDD)[b,c,d]	1,1'-(2,2-dichloroethylidene)- bis[4-chlorobenzene]	—Cl	—H	$-CHCl_2$
DDMU[c]	1,1'-(2-chloroethenylidene)- bis[4-chlorobenzene]	—Cl	None	$=CHCl$
DDMS[c]	1,1'-(2-chloroethylidene)- bis[4-chlorobenzene]	—Cl	—H	$-CH_2Cl$
DDNU[c]	1,1'-bis(4-chlorophenyl)ethylene	—Cl	None	$=CH_2$
DDOH[c]	2,2'-bis(4-chlorophenyl)ethanol	—Cl	—H	$-CH_2OH$
DDA[c]	2,2'-bis(4-chlorophenyl)- acetic acid	—Cl	—H	$-C(O)OH$
Some related insecticides				
Bulan®	2-nitro-1,1-bis (4-chlorophenyl)butane	—Cl	—H	$-CHC_2H_5$ NO_2
Prolan®	2-nitro-1,1-bis (4-chlorophenyl)propane	—Cl	—H	$-CHCH_2$ NO_2
DMC	4-chloro-α-(4-chlorophenyl)- α(methyl)benzenemethanol	—Cl	—OH	$-CH_3$
chlorobenzilate[e]	ethyl 4-chloro-α-(4-chlorophenyl)- α-hydroxybenzeneacetate			$-C(O)OC_2H_5$
chloropropopylate[e]	1-methylethyl 4-chloro-α (4-chlorophenyl)-α-hydroxy- benzeneacetate	—Cl	—OH	$-C(O)OCH(CH_3)_2$
methoxychlor[3]	1,1'-(2,2,2-trichloroethylidene)- bis[4-methoxybenzene]	$-OCH_3$	—H	$-CCl_3$
Perthane®	1,1'-(2,2,-dichloroethylidene)- bis[4-ethylbenzene]	$-C_2H_5$	—H	$-CHCl_2$
DFDT	1,1'-(2,2,2-trichloroethylidene)- bis[4-fluorobenzene]	—F	—H	$-CCl_3$

[a]Many of the compounds also exist as o,p'-isomers and other isomers in the technical grade and in the environment.
[b]Carcinogenicity discussed, evaluated, and quantitatively estimated in this report.
[c]Recognized metabolite of DDT in the rat, and a possible dicofol metabolite.
[d]As an insecticide, this compound has the International Organization for Standardization (ISO) approved name of TDE, it has been sold under the name of Rothane®; in metabolic studies the same compound has been referred to as DDD; as a drug, it is called mitotane.
[e]Common name approved by the ISO.

SOURCE: Adapted from World Health Organization, 1979.

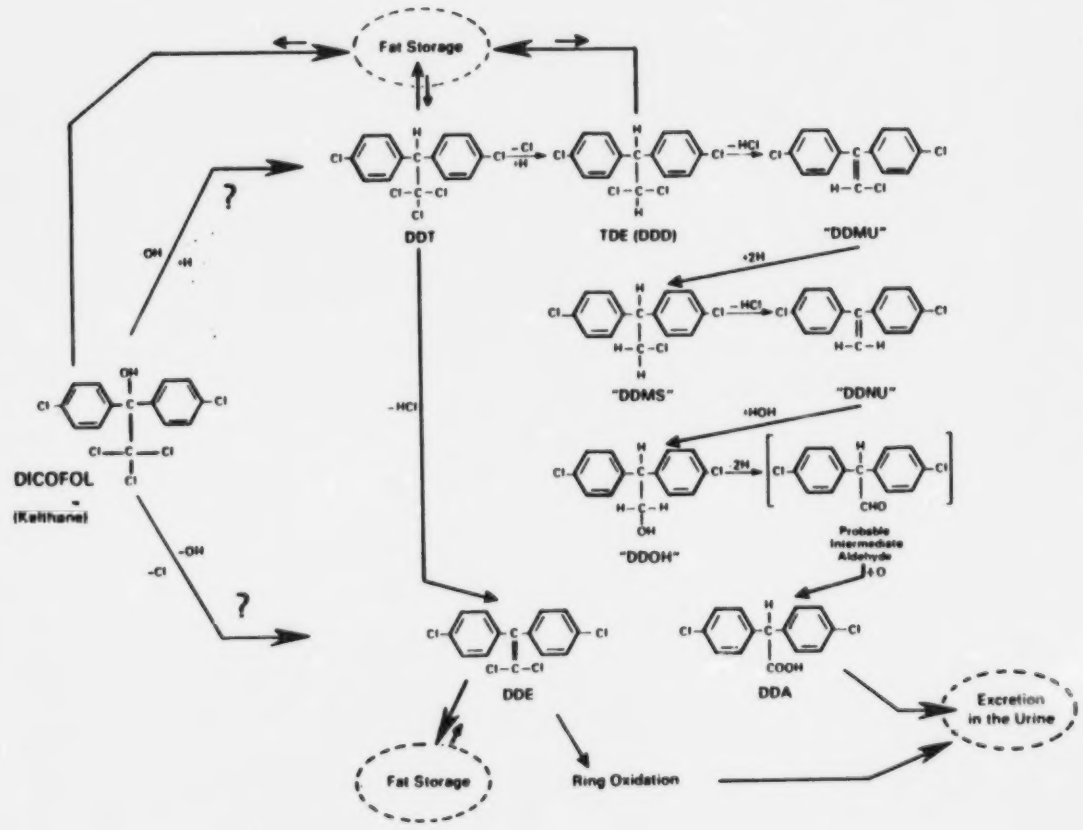

Figure 1. Theoretical metabolism of dicofol, and known metabolism of DDT, DDE, and DDD.

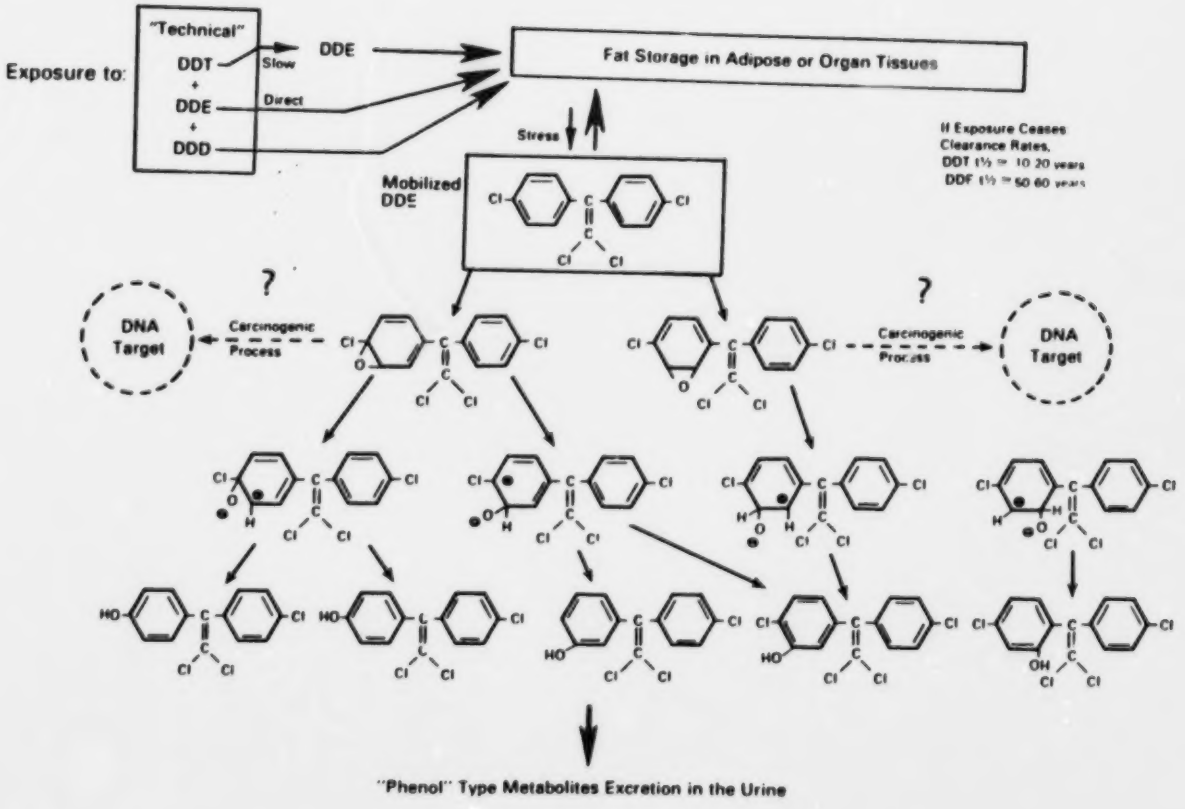

Figure 2. The ring oxidation of DDE.

genicity of these compounds in addition to that of dicofol. The present report considers the cancer potency of dicofol, DDT, DDE, and DDD. The Carcinogen Assessment Group (CAG) has reviewed the existing animal carcinogenicity data (mouse, rat, hamster, fish, dog, and monkey) and any available human cancer data on dicofol, DDT, DDE, and DDD. The Reproductive Effects Assessment Group (REAG) has reviewed the positive genotoxicity tests on DDT. As far as is known, no adequate studies have been done on the mutagenicity of dicofol. The present review by the CAG encompasses all available carcinogenicity studies of dicofol, DDT, DDE, and DDT available in the published literature as of January 1985, including a reconsideration of the mouse study previously used for risk estimation and from which a cancer potency estimate of 8.42 $(mg/kg/day)^{-1}$ was made (Tarjan and Kemeny, 1969). A current weight-of-evidence evaluation is made in this report of all adequate studies in order to determine the likelihood that these chemicals are carcinogenic. The CAG has determined that these chemicals are potentially carcinogenic to man, and therefore has selected the most appropriate carcinogenicity studies for determining the upper-bound estimate of the cancer potency.

Consideration is also given to the possible role of DDT in the mechanism of carcinogenesis, as either a complete carcinogen, a tumor promoter, a tumor initiator, or a genotoxic compound. Such mechanistic considerations could supply additional information as to the carcinogenicity of dicofol, DDT, DDE, and DDD.

2.2. BACKGROUND INFORMATION ON DICOFOL

Dicofol, also called Kelthane™, is a miticide used in the United States on berries, pome and stone fruits, citrus fruits, nut crops, cotton, field corn, seed crops, ornamental plants, greenhouse crops, and around domestic, commercial, and farm dwellings. Cotton and citrus fruits constitute the largest uses of

dicofol and account for about two-thirds of the two to three million pounds of dicofol (on an active ingredient basis) used each year in the United States.

Dicofol, a compound that is structurally related to DDT (see Table 1), is made in Israel by the Makhteshim-Agan Chemical Company and is distributed in the United States by Rohm and Haas. DDT and DDT-related compounds like dicofol are in current use in many countries, where the perceived benefits of these uses outweigh the anticipated risks. DDT was banned from use in the United States in 1972 by EPA Administrator William Ruckelshaus. The ban was based on the bioaccumulation of DDT, DDE, and DDD, which had been found to produce deleterious effects in birds, fish, and other organisms. While the ban was not based on demonstrated effects to public health, there was concern that such effects might exist, on the basis of known human exposures to DDT and the fact that some studies at that time indicated that DDT produced liver and lung tumors in mice. In addition, there was concern that DDT might have reproductive effects in humans, since reproductive effects had been noted in lower animals, especially birds.

DDT and DDE are both readily absorbed into the human body in direct proportion to dietary exposure (WHO, 1979). An estimate of the extent of such absorption in milligrams incorporated per kilogram of body weight (ppm) is: $\log C_1 = 0.7 \log I + 1.3$, where I is the average dietary intake in mg/kg/day. The residues of these compounds are retained throughout the body, usually in proportion to the percentage of fat in an organ and in depot lipids. The body burden is long-lived, with clearance rates for man (in half-lives) of as long as 10 to 20 years for DDT and 60 to 70 years for DDE. It is clear that once humans are exposed, such residues are retained for long periods in the body, with subsequent exposures adding to the preexisting body burden. These residues are thus of concern in the United States in spite of the 1972 ban on DDT,

since the populace is still being exposed to the residues, which continue to add to the preexistent DDT/DDE body burden.

The pervasiveness of DDT, DDE, and DDD residues in geographic areas in which DDT formulations have been used is well known. Due to the striking similarities in chemical structure between dicofol and DDT, this pervasiveness presumably holds for dicofol as well, but this is not known at this time. It is suggested in Figure 1 that metabolic interrelationships may exist among dicofol, DDT, and DDT metabolites. In Figure 2 a scheme is proposed in which possible carcinogenic intermediates could occur in DDE degradation. A long half-life in soil allows incorporation of DDT and/or DDE residues into crops, which in turn are ingested by the human population. DDT and DDE residues are also passed from the simpler organisms up the food chain to higher organisms, such as wild game, which are eaten by the human population, further adding to the body burden. Thus, residues of dicofol, DDT, DDE, and DDD in food present an environmental problem.

A five-generation mouse carcinogenicity study conducted in Hungary (Tarjan and Kemeny, 1969) was previously selected for hazard evaluation by the CAG from five different positive studies on DDT. This study was used by the CAG to estimate the upper limit of cancer potency for DDT, if DDT is a human carcinogen (U.S. EPA, 1980a). At an average lifetime dietary dose of 0.45 mg/kg body weight/day, a cancer potency (q_1^*) for DDT was estimated to be 8.422 $(mg/kg/day)^{-1}$, based on malignant (but not metastasizing) lung tumors in BALB/c mice.

3. ANIMAL STUDIES - QUALITATIVE DISCUSSION

3.1. ANIMAL STUDIES ON THE CARCINOGENICITY OF DICOFOL

A 2-year bioassay was performed by the National Cancer Institute (NCI) on technical-grade dicofol in B6C3F1 mice (NCI, 1978a). Dicofol was mixed into the feed at 264 and 528 ppm for male mice and 122 and 243 ppm for female mice. The animals were dosed with dicofol for 78 weeks, followed by 15 weeks of observation until terminal sacrifice. There were 50 mice of each sex per dose group.

B6C3F1 mice of both sexes exhibited no specific nonneoplastic lesions, and no increased mortality was observed in either males or females fed dicofol. Female mice showed a mild decrease in body weight at the high dose (243 ppm) from 37 weeks to termination, and showed an even milder decrease at the low dose (122 ppm) from 41 weeks to termination.

Female B6C3F1 mice, as compared with controls, did not respond to dicofol with excess tumors of any kind. The neoplastic responses for male B6C3F1 mice were positive and were as follows:

	Control	Low dose	High dose
1. Male mice at start	20	50	50
2. Male mice examined histologically	18	48	47
3. Male mice with primary tumors of any tissue kind, including benign and malignant	5	34	38
4. Hepatocellular adenomas (male)	0	1	1
5. Hepatocellular carcinomas (males)	3	22	35
6. Combined hepatocellular tumor response (males)	3	23	36

The dose-response trend of the combined liver tumors in the males is significant at the $p < 0.001$ level, with the low-dose liver tumor incidence increased over controls at $p = 0.0035$ and the high-dose incidence increased over controls at $p < 0.001$. These statistical tests suggest a significant quantitative response based upon a highly significant qualitative response which was characterized by a high proportion of malignant hepatocellular tumors in male B6C3F1 mice.

Osborne-Mendel rats were tested also with technical-grade dicofol (85% to 90% active ingredient) at a rate of as high as 942 ppm (= 122 mg/kg body weight/day). No excess tumors were observed in treated rats as compared with control rats.

3.2. ANIMAL STUDIES ON THE CARCINOGENICITY OF DDT

3.2.1. Mice

Nine dietary feeding studies have been conducted on DDT in mice. These carcinogenicity bioassays were done in the USSR, Italy, England, the United States, India, and Hungary on a total of 4,333 mice of various strains (Table 2). Only one of these studies (NCI, 1978b) indicated no excess tumors due to DDT exposure, while six other studies indicated excess liver (and, in two studies, lung) tumors in the mouse. In the one negative study, mice were dosed for a relatively short period of 78 weeks.

The general pattern of the carcinogenic response to DDT in mice is described below and qualitatively summarized in Table 2. Quantitative cancer potency estimates from adequately conducted studies are presented in Chapter 7.

Both benign tumors (hepatocellular adenomas) and malignant tumors (hepatocellular carcinomas) were observed in the six positive liver tumor studies. Benign and malignant lung tumors were observed in the two multigeneration studies. Generally, the mouse tumors were not life-threatening in that dosed mice lived as long as control mice and as long as expected for the various

TABLE 2. SUMMARY OF DDT DIETARY CARCINOGENICITY STUDIES IN MICE

Study (in order of increasing maximum dose)	Mouse strain	Total no. of dosed mice	Maximum length of treatment (weeks)	Maximum dosage (mg/kg/day)	Evidence of carcinogenicity[a]	Tumor location	State of malignancy[b] (benign/malignant)	Comments
1. Shahad et al., 1973	A-strain	234	lifetime[c]	0.15	+	Lung	Benign	--
2. Tarjan and Kemeny, 1969	BABL/c	683	lifetime[c]	0.45	+	Lung/ Leukemia	Benign & malignant	Used in 1980 Water Criteria Document to determine cancer risk from DDT
3. Walker et al., 1972	CF-1	60	112	15	+	Liver	Benign & malignant	--
4. Thorpe and Walker, 1973	CF-1	33	110	15	+	Liver	Benign & malignant	--
5. Kashyap et al., 1977	Swiss/ Bombay	60	80	15	+	Lymphomas/ Lung/Liver	Malignant	--
6. Innes et al., 1969	C57BL C3HxAKR F_1	72	85	21	+	Liver	Benign	--
7. NCI, 1978b	B6C3F1	200	78[d]	26.3	-	--	--	--
8. Terracini et al., 1973	BALC/c	227	135[e]	37.5	+	Liver	Benign (& malignant?)	Two-generation study; malignancy not well characterized or described
9. Turusov et al., 1973	CF-1	2,764	lifetime[c]	37.5	+	Liver	Benign (with only a few malignant)	Six-generation study; tumor yield about the same for each generation

[a]A "+" = a statistically significant ($p \leq 0.05$) increase in the number of mice with tumors, as compared with controls; a "-" = no excess number of mice with tumors as compared with controls ($p > 0.05$).
[b]No tumors observed in any of the studies were metastatic.
[c]A multigeneration study in which animals treated with DDT were exposed in utero until death.
[d]Included 78 weeks of dietary exposure plus 15 weeks of observation, with sacrifice at 93 weeks.
[e]A two-generation study in which each generation was observed from week 5 until week 140.

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strains tested.

The most common response to DDT in mice occurred in the liver. Heterogeneous cellular responses in mouse liver were observed, indicating various stages of stimulated growth and tumorigenicity, as well as certain necrotic conditions, seen especially at higher DDT dose levels. The livers first showed reversible focal hyperplasia. With continued DDT exposure, some of these foci are known to be able to convert to nodules. The nodules resulting from DDT varied in size and cellular organization, but were most often composed of solid cords of closely packed cells one to two cells thick. These cells differed little from normal hepatocytes. The larger nodules compressed the surrounding parenchyma. More malignant states were also observed in the mouse livers and were classified as hepatocellular carcinomas. These DDT-induced lesions were morphologically organized in wide trabeculae that formed papillary, glandular, and sometimes whorl patterns. Occasionally, anaplastic regions were observed, arranged in rosettes. Necrotic or hemorrhagic areas were observed along with cystic areas. Invasiveness was limited locally in the liver and lung, and dissemination followed by metastasis was not observed in any of the studies.

These studies indicate either that DDT is acting in the mouse liver and lung as a complete carcinogen (that is, as both an initiator and a promoter) or that laboratory mice are already inherently initiated and are thus uniquely sensitive to a compound such as DDT, which has well-documented promotion potential (Periano et al., 1975; Scribner et al. 1983; Hilpert et al., 1983, Ito et al., 1982, 1983; and discussions and references in Pitot and Sirica, 1980). In either case, however, DDT by itself causes liver and lung tumors in mice, a finding which indicates that there is a potential for the same reaction in humans.

3.2.2. Hamsters

Syrian Golden hamsters were fed 0, 125, and 500 ppm technical-grade DDT for their lifetimes (Cabral et al., 1982a). Calculated doses were 0, 10, 20, and 40 mg DDT/kg body weight/day. No statistical increase in any specific tumor type was observed. It is especially relevant to note that, contrary to the mouse response, no increase in liver or lung tumors was observed. Thus, although the doses given to the hamsters were comparable to the doses in the mouse studies, no tumors were produced in the hamsters, thereby indicating that the hamster is refractory to DDT in the diet.

In another study, DDT or DDE was incorporated into the diet of hamsters (Rossi et al., 1983). DDE was active in producing liver tumors (neoplastic nodules, not carcinomas); DDT did not produce tumors. This observation is interpreted to mean that the metabolite of DDT (that is, DDE) could be the active agent (Rossi et al., 1983). It should be noted that in mice, both DDE (liver tumors) and DDD (lung tumors) are oncogenic (Tomatis, 1974).

It is likely that DDT is not carcinogenic in hamsters, since it only accumulates in the hamster's body tissues and does not readily undergo the conversion from DDT to DDE (Gold and Brunk, 1983). In contrast, mice and humans readily convert DDT to DDE and DDT to DDD (WHO, 1979). The hamster bioassay data indicate that the DDT metabolites, DDE and DDD, are carcinogenic, but that DDT is not carcinogenic in the hamster.

3.2.3. Rats

Eight studies have been reported in which DDT was fed to rats in the diet. The carcinogenicity results of these studies are presented in Table 3. A total of 1,095 rats of various strains at various laboratories were exposed to DDT in these studies. Three of the studies were positive for DDT-induced tumors at doses of $\geq$ 25 mg/kg body weight/day, while one study (NCI, 1978b) had negative

TABLE 3. SUMMARY OF DDT DIETARY CARCINOGENICITY STUDIES IN RATS

Study (in order of increasing maximum dose)	Rat strain	Total no. of dosed rats	Maximum length of treatment (weeks)	Maximum dosage (mg/kg/day)	Evidence of carcinogenicity[a]	Tumor location	State of malignancy[b] (benign/malignant)	Comments
1. Treon and Cleveland, 1955	Carworth	240	104	1.2	-	--	--	--
2. Kimbrough et al., 1964	Sherman	75	40	2	-	--	--	--
3. Deichmann et al., 1967	Osborne-Mendel	60	104	10	-	--	--	--
4. Radomski et al., 1965	Osborne-Mendel	60	104	12	-	--	--	--
5. Rossi et al., 1977	Wistar	72	152	25	+	Liver	Benign	At 0 and 25 mg/kg/day, hepatomas[c] 0/67 and 24/50; DDT compared to phenobarbital in same study; both produced only nodules; TBA invariant.
6. Cabral et al., 1982b	MRC Portion (Wistar-derived)	196	120	25	+	Liver	Benign	No. of TBA constant with dose; only females affected; at 0, 6.3, 125, and 25 mg/kg/day, hepatomas were 0/38, 2/30, 4/30, and 7/38, i.e., mild response
7. NCI, 1978b	Osborne-Mendel	200	78[d]	26.5	-	--	--	--
8. Fitzhugh and Nelson, 1947	Osborne-Mendel	192	104	40	+	Liver	Benign	Centrilobular necrosis observed

[a]A "+" = a statistically significant ($p \leq 0.05$) increase in the number of rats with tumors, as compared with controls; a "-" = no excess number of rats with tumors as compared with controls ($p > 0.05$).
[b]None of the tumors observed were metastatic.
[c]Hepatomas are generally defined in this document as benign liver tumors, sometimes referred to as "hepatocellular adenomas." "Hepatomas" is used where the authors use this term to refer to liver tumors.
[d]78 weeks dosing with DDT, plus an additional 35 weeks for observation.

TBA = total tumor bearing animals; denotes tumors of any type.

results. The time period of dietary exposure was comparatively short for the one negative study (78 weeks). In all three of the positive studies, only benign liver tumors were produced, with the total of tumor-bearing animals invariant among treated and control groups. Mortality was not increased in the dosed groups. As seen in the qualitative presentation in Table 3, doses of less than 25 mg/kg/day in the rat produced no excess tumor response of any kind --a finding that suggests the existence of an experimental threshold dose level.

3.2.4. Fish

Trout, which normally live as long as 5 to 6 years, were exposed to DDT at 75 ppm in the diet for 20 months. Trout fed DDT exhibited hepatomas (author's terminology) at 20 months, with an incidence rate of 11/30 (37%), whereas the incidence in controls at 20 months was 0/400 (0%) (Halver, 1967). A second experiment was performed with the same protocol and showed similar results.

On the basis of the above evidence, it is concluded that dietary exposure to DDT causes carcinogenesis in trout.

3.2.5. Dogs

Dogs were exposed to DDT in the diet at concentrations of 0 (2 dogs), 400 (2 dogs), 2,000 (4 dogs), and 3,200 ppm (14 dogs) (Lehman, 1952 and 1965). This was equivalent to dosing rates of 0, 10, 50, and 80 mg DDT/kg body weight/day. All of the 14 dogs at 3,200 ppm died of toxicity. At 2,000 ppm, 2 of 4 dogs died of toxicity. The remaining 6 dogs survived to the time of sacrifice (39 to 49 months), which is approximately 30 to 40 percent of the life expectancy of the dog.

None of the dogs dying of toxicity, and none of the dogs surviving to planned sacrifice, had excess tumors upon autopsy (0/18). Liver damage was

observed, but no liver tumors were evident. Thus, in the dog, DDT may not be carcinogenic at maximum tolerated doses during 30 to 40 percent of the animals' lifetimes. Such a conclusion, or any other conclusion for that matter, would be questionable since so few dogs survived the toxicity of DDT.

3.2.6. Monkeys

In two studies (Adamson and Sieber, 1979 and 1983), monkeys from an NCI colony were treated with a control diet or a control diet containing technical-grade DDT five times/week at 20 mg/kg/body weight/day. Positive controls were given aflatoxin B in the diet. The negative control monkeys exhibited a baseline tumor rate of 3.2 percent. The animals treated with aflatoxin B showed an overall tumor rate of 40 percent, with one-half of the tumor-bearing animals developing liver tumors. This result indicated that the monkeys from the NCI colony could, if treated with a known hepatocarcinogen, produce liver tumors as early as 5 years after the start of dosing.

In these studies, DDT did not produce excess tumors of any kind in monkeys. The monkey species, which included rhesus, cynomolgus, African green, and bush babies, did not produce a carcinogenic response in 134 months, which is approximately one-third of a rhesus monkey's lifetime. This negative finding in the monkey is seemingly corroborated by another study of monkeys by Durham et al. (1963), in which no DDT-induced tumors were found in 7.5 years in rhesus monkeys at a DDT dose rate as high as 100 mg/kg/day. These results suggest that DDT is not carcinogenic in monkeys; however, the studies were not conducted for long enough periods for a firm determination of noncarcinogenicity to be made.

3.3. ANIMAL STUDIES ON THE CARCINOGENICITY OF DDT METABOLITES, DDE AND DDD

3.3.1. DDE

In a study conducted by the NCI (1978b), B6C3F1 mice were fed 148 ppm (19.2 mg/kg/day) and 261 ppm (34 mg/kg/day) DDE for 78 weeks, with 15 additional

weeks of observation before termination. DDE in the females caused a DDE-dependent loss in weight as early as 10 weeks; the male weights were unaffected. The mortality curve (increased deaths before termination of the experiment) in the female mice was also affected by DDE ($p < 0.001$), whereas male mortality was not affected. Hepatocellular carcinomas were observed in mice of both sexes, with the strongest response occurring in the females. The incidences of carcinoma in the control, low-, and high-dose animals, respectively, were as follows: females, 0 (0%), 19/47 (40%), and 34/48 (71%); males 0/19 (0%), 7/41 (17%), and 17/47 (36%).

In a parallel NCI study (1978b), Osborne-Mendel rats did not respond with tumors when fed DDE in a 2-year bioassay. The rats did exhibit liver involvement in the form of centrilobular necrosis and fatty metamorphosis.

In a study by Tomatis et al. (1974b), CF-1 mice were fed 250 ppm (32.5 mg/kg/day) DDE for 130 weeks. The female mice treated with DDE showed increased hepatomas (authors' terminology) (54/55 vs. 1/90 in controls) as well as early appearance of hepatomas, thereby indicating that DDE-induced hepatomas may have been life-threatening. Male CF-1 mice responded similarly (39/53 vs. 33/98 in controls) and died earlier with hepatomas. The hepatomas were largest in size and occurred with the greatest multiplicity (hepatomas/mouse) in DDE-treated mice as compared with control mice. Residue data from autopsies performed on the CF-1 mice showed that DDE was retained in the liver to a degree second only to its rate of retention in body fat and in liver tumors (at about the same levels). DDE residues also occurred in normal livers at about the same levels as in tumorous livers, thereby indicating that the residual presence of DDE is not, in and of itself, a sufficient cause for carcinogenesis in mice.

DDE was also tested for carcinogenicity in the hamster (Rossi et al., 1983). At doses of 500 ppm (40 mg/kg/day) and 1,000 ppm (80 mg/kg/day), DDE

in the diet of hamsters caused neoplastic nodules (hepatomas) in males (4/39 and 6/39) and in females (7/30 and 8/39). These hamster liver tumors had a relatively long latency period of more than 76 weeks. DDT did not produce tumors in hamsters at 500 and 1,000 ppm (Cabral et al., 1982a; Rossi et al., 1983).

These DDE studies indicate that the Osborne-Mendel rat is refractory to DDE-induced carcinogenesis, but that the mouse (B6C3F1 and CF-1 strains) and hamster (Syrian Golden) are susceptible. Since humans absorb and produce DDE in the metabolism of DDT, and since DDE has a higher affinity for body fat than DDT, and appears to be carcinogenic in the hamster, whereas DDT is not, it is relevant to consider the human risks of DDE. An upper-limit estimate of the cancer potency of DDE in humans is presented in Section 7.7.

3.3.2. DDD

An NCI report on a 2-year study in which Osborne-Mendel rats were fed DDD indicated no significant excess liver tumors in either sex at doses of 850 to 3,294 ppm (NCI, 1978b). These rats did, however, respond with some thyroid adenomas and carcinomas in the follicular cells and C-cells at these high doses. The C-cell response was only marginal, and neither of the thyroid responses showed a trend with DDD dose. The past wide variation in rat historical controls for these tumor types (especially in older animals) confounds the interpretation of these results.

In the same NCI study, B6C3F1 mice were dosed with DDD at 411 and 822 ppm. No significant excess tumors were observed, except for hepatocellular carcinomas [controls 2/11 (18%), low-dose 12/44 (27%), and high-dose 14/50 (28%)]. This liver response was also judged by the NCI to be insignificant, since controls had responded with excess tumors of up to 20% in the past.

In another feeding study of CF-1 mice given DDD at 0 and 250 ppm, it was

found that lung tumors, as well as liver tumors, were induced by DDD (Tomatis et al., 1974b). Lung tumors in male mice increased from 53/98 (54%) in controls to 51/59 (86%) at 250 ppm; and in female CF-1 mice, lung adenomas increased from 37/90 (41%) to 43/59 (73%). Liver tumors in males were increased from 33/98 (34%) to 31/59 (52%), whereas female CF-1 livers were unaffected. DDD caused only a slightly accelerated increase in the mortality of mice with hepatomas (authors' terminology), whereas DDE caused markedly early deaths of CF-1 mice with hepatomas, and DDD + DDE (same total level, 250 ppm) caused an intermediate acceleration in the mortality of mice with hepatomas. DDD did not cause an increase in the total number of tumor-bearing animals, nor did it cause an increase in the multiplicity of tumors. These data from Tomatis et al. (1974) suggest that DDD is only a mild carcinogen in CF-1 mice.

No cancer bioassays of DDD in hamsters have been reported. Such studies would be helpful in determining the possible carcinogenicity of DDT as compared with DDT metabolites such as DDD.

4. EPIDEMIOLOGIC CONSIDERATIONS

There are no known epidemiologic studies on dicofol.

The effects of DDT on humans have been reviewed previously (IARC, 1974; WHO, 1979; U.S. EPA, 1980a). It was the consensus of these reviews, which included several prospective and case-control studies, that the data were based on studies that were too limited and/or too short for any conclusions to be made as to carcinogenesis. No further review of the literature on DDT epidemiology has been conducted since 1980.

It is, therefore, concluded, due to a lack of evidence, that epidemiology does not factor into the present weight-of-evidence consideration for the carcinogenicity of DDT, and, by comparison, dicofol.

5. ADDITIONAL EVIDENCE OF CARCINOGENICITY

5.1. DDT PROMOTION OF HEPATOCARCINOGENESIS

5.1.1. Definitions of Tumor Initiation and Tumor Promotion Processes in Chemical Carcinogenesis

Since the possibility exists that dicofol, DDT, and/or DDT metabolites are carcinogenic to humans, it is germane to further examine the carcinogenic properties of these substances. The mechanistic investigations to date have been conducted primarily on DDT, mainly because of the ubiquitous usage of DDT worldwide and the known body burdens of DDT residues due to direct contact and to movement up the food chain. It is assumed that dicofol, because of its structural similarity to DDT, might behave similarly to DDT in the stages of the carcinogenic process.

Cancer is essentially a lack of coordination and temporal control of cellular maintenance and growth in a normal field of cells. When the loss of control is persistent, the result is an evolving neoplastic process, followed by tumorigenesis. The whole process, if caused by a chemical agent, is called chemical carcinogenesis. Chemical carcinogenesis has been divided conceptually into two distinct sequential events: initiation and promotion.

Tumor initiation is thought to be an oncogenic process in which some of the cells in a normal field of cells are altered by changing (often by mutation) the cellular DNA function. The process of tumor initiation is thought to be essentially an irreversible, additive, and nonthreshold set of events (Pitot and Sirica, 1980).

Tumor promotion is thought to be a process in which the usage of the cellular genetic information is altered by the imposition of perturbation events that disrupt the normal negative cellular control mechanisms. Such perturba-

tions cause uncoordinated and untimely growth events, which are usually controlled in a normal field of cells by cellular contact inhibition. If such growth events are persistent, local hypertrophy and hyperplasia result, with the previously initiated cells demonstrating a relative growth advantage.

5.1.2. Possible Mechanism of Tumor Promotion in the Target Tissue - Liver

Promotion has been adequately demonstrated in the skin and liver, and has been implicated in the mammary gland, bronchus, esophagus, and bladder (Pitot and Sirica, 1980; Pitot, 1982). Some, but not all, of the early-forming neoplasms progress to fully grown tumors. While promotion in the liver is thought to be reversible in the early stages, such promotion tends to change, with time of exposure, to an essentially irreversible phase characterized by uncontrolled propagation of the affected cells, leading to various stages of malignancy. The degree of "promoted" malignancy can vary from benign, noninvasive, circumscribed tumors to malignant tumors, which can be locally invasive, regionally disseminating, or metastatic throughout the body.

Unlike initiation, the process of promotion is thought by some to be a threshold set of events; that is, there would be a level of exposure below which tumor promotion would not occur (Pitot, 1982; Boutwell, 1964). In the liver, the initial phase of promotion is thought to be reversible because cessation of repeated exposure to the chemical agent, such as DDT, causes reversal of the foci both in size and in number (Schulte-Hermann et al., 1982; Ito et al., 1982, 1983). Mechanistically, this initial tumor promotion phase for DDT is thought to be brought about by dissolution of DDT into the cell membrane and disruption of cell membrane-mediated events, including cell-to-cell communication (Madhukar et al., 1983; Williams, 1981). Continued exposure to a chemical such as DDT can then release a sufficient number of cells from contact inhibition so that a majority of the affected cells would be isolated from normal cells.

The neoplasm would progress in stages to more malignant states by becoming progressively more independent of promoter exposure (Williams, 1981). When these later events take place, propagation of the tumor is essentially irreversible, since it has escaped integrated organismic control (Tomatis and Turusov, 1975). It has been proposed that the main events of DDT promotion are solely epigenetic in the liver, since DDT has not been found to be genotoxic, i.e., to cause unscheduled DNA synthesis, in mouse, rat, and hamster hepatocytes (Maslansky and Williams, 1981). The explanation of these negative findings in hepatocytes, with respect to the positive genotoxic tests reported in Section 5.2, is not yet clear.

5.1.3. Tumor Promotion as Additional Evidence that DDT, DDE, and DDD Are Carcinogenic in Rats

It is apparent that DDT, DDE, DDD, and dicofol are carcinogenic in various mice strains (see Chapter 3). This could mean that the mice are already initiated by DDT (or DDE or DDD), or it could mean that in mice DDT is a complete carcinogen, i.e., an initiator and a promoter. In either case, DDT exhibits promoter activity, and presumably, dicofol, DDE, and DDD can too.

Liver tumors have been induced by DDT in the rat by the classical promotion protocol: a short dietary exposure of a known rat liver initiator, 2-acetylaminofluorene (2-AAF), followed by a lifetime dietary exposure to DDT. Rats receiving only a short dietary exposure of 18 days of 0.02% 2-AAF in the diet had a tumor incidence pattern similar to sham-treated control rats, whereas rats treated for 18 days with 0.02% 2-AAF, followed by 0.05% (= 40 mg/kg/day) of technical-grade DDT in the diet, showed a significant liver tumor response (Peraino et al., 1975). At this average dose of 40 mg DDT/mg body weight/day, 45 percent of the rats had tumors (adenomas or carcinomas) at 100 days, while the average liver tumor load was 0.6 tumors per liver; at 300 days, approximate-

ly 80 percent of the rats had tumors (controls = 30 percent), while the average liver tumor load was 2.5 tumors per liver.

In another DDT promoter study in rats, DDT caused the acceleration of 2-acetamidophenanthrene (2-AAP)-initiated mammary tumors and ear duct tumors in males, but was negative for liver tumors (Scribner and Mottet, 1981). In yet another rat study from the same laboratory with 2-acetylaminofluorene (2-AAF) or 2-acetamidophenanthrene (2-AAP) as initiators, DDT as a promoter caused foci formation in the liver with elevated gamma-glutamyltranspeptidase staining, a marker for the preneoplastic state in liver (Scribner et al., 1983). DDT has been compared to phenobarbital, a known liver promoter, and was found to be similar to phenobarbital in its promotion characteristics (Pitot, 1982; Scribner et al., 1983; Peraino et al., 1975).

Finally, rats initiated with trans-4-acetylaminostilbene were found to have precancerous conditions in many tissues, including the liver, but only mammary tissue responded with tumors when promoted with exposures to DDT in the diet (Hilpert et al., 1983). Such tissue specificity indicates that the conjunction of initiator and promoter is important to the organ localization of tumors, and emphasizes the importance of identifying tumor promotion potential in a chemical such as DDT.

It is concluded that DDT acts as a complete carcinogen in the mouse, causing adenomas and carcinomas primarily in the liver and also in the lung in some multigeneration studies. The possibility cannot be ruled out that DDT is a strong promoter only, and that the mouse liver tumors had already been initiated by intrinsic, vertically transmitted factors. The problems in interpreting the mouse liver tumor response have been reviewed by Doull et al. (1983).

It should be pointed out that (1) the tumors produced in the mice were never metastatic; (2) the total numbers of tumor-bearing mice were usually not

very different among control and DDT-treated groups--a factor which indicates that any increase of mice with liver tumors was at the expense of tumors of other types (i.e., DDT is causing only a shift in tumor pattern); and (3) the liver tumors were usually discovered late in the lifetimes of the test mice, and appeared not to be life-threatening. These observations suggest that chemical carcinogenesis (tumor initiation, promotion, and propagation) due to DDT in mice is limited and does not progress to more advanced malignant states.

In the eight studies (Table 3) done on rats, five were negative for carcinogenicity and three were positive with hepatomas (benign liver tumors). The same three tumor characteristics described for mice in the previous paragraph also apply to rats. It appears that at higher doses, DDT can be a promoter of benign hepatomas in rats. In most of the studies, however, DDT did not produce liver tumors. On the basis of the above results, the CAG has concluded that DDT has carcinogenic potential in the rat based on the limited positive oncogenic results observed at or higher than 25 mg/kg/day in the rat diet.

5.2. GENOTOXICITY OF DDT, DDE, AND DDD

DDT has been tested extensively for genotoxicity, and both positive and negative results were obtained, thereby precluding an unequivocal determination of genotoxicity for DDT. In the mouse dominant lethal tests conducted by Epstein and Schafner (1968) and Wallace and Knights (1976), no increase in mortality was observed, nor was there an increase in visible or lethal mutations after five generations. Mutagenesis in the wasp was also found to be negative (Grosch and Valcovic, 1969). Negative evidence for an effect of DDT on unscheduled DNA synthesis in human fibroblasts in culture has been shown (Ahmed et al., 1977), as well as negative evidence in mouse, rat, and hamster hepatocytes for unscheduled DNA synthesis (Maslansky and Williams, 1981; Probst et al., 1981). Further, DDT was found not to be mutagenic <u>in vitro</u> in rat liver epithe-

lial cells (Williams, 1979). Human fibroblast cells (also in G.M. Williams' laboratory) were not genotoxically effected in a rat hepatocyte-mediated assay (Tong et al., 1981), and did not produce chromosome aberrations in cultured human lymphocytes (Lessa et al., 1976). In the classic Ames Salmonella typhimurium systems, DDT was not mutagenic with or without the S-9 metabolizing cell-fraction preincubation (Van Dijck and Van de Voorde, 1976; Marshall et al., 1976; Planche et al., 1979). Lastly, no genetic effects were found in yeast (Fahrig, 1974).

In contrast to the above negative studies, DDT induced positive mutagenicity in V79 Chinese hamster cells in vitro (Bradley et al., 1981). Chromosome aberrations in cultured human lymphocytes were observed in two studies (Rabello et al., 1976; Preston et al., 1981). DDT was shown to increase the frequency of sister chromatid exchanges in V79 and in CHO cells (Ray-Chaudhuri et al., 1982). In one study (Kubinski et al., 1981), DDT was reported to interact directly with DNA. In another study, however, in which the metabolizing system was lacking (Griffin and Hill, 1978), DDT did not interact with DNA.

DDE, a contaminant and putative metabolite of dicofol, was found to have positive mutagenic effects in mouse lymphoma cells (L5178Y cells) and Chinese hamster cells (V79 cells) [International Commission for Protection Against Environmental Mutagens and Carcinogens (ICPEMC)]. ICPEMC (1984) reported that positive genotoxic effects were also found in mammalian cytogenetic assays of DDE and DDD.

In a recent review of the above genotoxicity studies, ICPEMC arrived at the conclusion that the genotoxicity studies of DDT do not present either clearly positive or clearly negative findings (ICPEMC, 1984). Further, Dr. Lawrence R. Valcovic of the Reproductive Effects Assessment Group (REAG) has been requested by the CAG to review the studies on the genotoxicity of DDT

(memorandum from James W. Holder, CAG, to Peter E. Voytek, REAG, 8/8/84). Dr. Valcovic is in agreement with the conclusions of the ICPEMC report and has stated that the positive genotoxicity data (if proven to be valid) suggest a potential genotoxic component for DDT (memorandum from Lawrence R. Valcovic, REAG, to James W. Holder, CAG, 8/30/84.)

Although the relative strength of such a genotoxic component is not known, the gene toxicity data are strong enough to indicate that DDT, DDE, and DDD have the capacity to initiate the carcinogenic process. This genotoxic component, combined with its tumor-promotion activity (reviewed in Section 5.1.3.), indicates that DDT is able to act as a total carcinogen in some animal biotest systems--a statement that is borne out by the mouse biotest data presented in Table 2. Although the possibility exists that mice inherently possess the proclivity for tumor formation resulting from exposures to chlorohydrocarbons (Weisburger, 1982; Doull et al., 1983), the genotoxicity data nevertheless suggest that DDT could have an initiation potential in humans and, for this reason, must be regarded as a complete carcinogen with characteristics of a no threshold, additive, and irreversible type of dosimetry. The CAG has therefore estimated the cancer potency of DDT, DDE, DDD, and dicofol under the assumption that these compounds are complete carcinogens.

6. WEIGHT OF EVIDENCE THAT DDT IS A CHEMICAL CARCINOGEN

6.1. POSSIBLE CHEMICAL CARCINOGENICITY TO BIOTEST ANIMALS AS A RESULT OF DDT EXPOSURE

Results from biotests in various strains of mice (Table 2) indicate that eight of nine studies were positive, with the types of oncogenic response being mostly liver tumors, and sometimes lung tumors and leukemias. Both carcinomas and adenomas were observed in the eight positive studies in a wide dose-rate range of 0.45 to 37.5 mg DDT/kg body weight/day of dietary exposure. On the basis of these results, the CAG feels that adequate evidence exists for the carcinogenicity of DDT in the mouse.

Results from biotests in various strains of rats (Table 3) indicate that three of eight studies were positive, with positive oncogenesis occurring only at rather high dose-rates of dietary exposure ($\geq$ 25 mg DDT/kg body weight/day). Osborne-Mendel rats did not respond at 26.5 mg/kg/day but did respond at 40 mg/kg/day. The oncogenic responses in all three of the positive rat studies were in the liver, in the form of benign tumors only (often referred to as hepatomas). The total number of tumor-bearing animals (TBA) in rats did not change under DDT exposure, as compared with controls, and no DDT-induced early mortalities were observed; only a change in tumor pattern was evidenced (constant number of TBA) and not life-threatening effects from DDT. These results indicate only limited evidence for carcinogenicity in the rat.

The hamster was refractory to biotest DDT doses of up to 40 mg/kg/day. This result could be due to the slow metabolic conversion of DDT to DDE. Only DDE (not DDT) produced benign liver tumors, but did so only at higher doses (40 and 80 mg DDE/kg/day). The DDE response in liver is a marginally significant response (treated animals versus controls, $p = 0.05$) but showed no trend

with dose. These results in the hamster are judged as evidence for no carcinogenicity for DDT and only limited evidence for DDE.

Trout were exposed to DDT concentrations of 75 ppm in the diet for one-third of their lifetimes. A significant response of 37 percent (11/30) in the form of benign hepatocellular adenomas was observed in treated fish, as compared to no responses (0%) in the 400 trout controls. The benign oncogenic response after a limited exposure period to DDT is judged to constitute limited evidence for carcinogenicity in the fish. Further, fish do not represent a close biological surrogate for humans, and thus, the qualitative weight of this piscine evidence is unclear at the present.

Dogs were treated with 10, 50, and 80 mg DDT/kg body weight/day in the diet. All of the dogs at 80 mg/kg/day (14 dogs) died of toxicity, and one-half of the dogs (2 of 4) at 50 mg/kg/day died of toxicity. The rest (4 dogs) were treated for about one-third of the average dog lifetime. No tumors were observed. The data are based on a small number of dogs that were treated for only part of their lifetimes, and therefore are judged to constitute inadequate evidence that DDT is not a carcinogen.

Twenty-four monkeys treated at 20 mg/kg/day for approximately one-third of their lifetimes showed no excess tumors, while rhesus monkeys in another study treated at dose rates of up to 100 mg/kg/day for 7.5 years did not produce excess tumors. The results of both of these primate biotests taken together, form limited evidence for no effect from DDT.

The metabolites of DDT, namely DDE and DDD (and possibly the metabolites of dicofol, as shown in Figure 1), can produce oncogenesis. A ring epoxide in the oxidation of DDE (Figure 2) could be a candidate for a carcinogen because of its structural similarity to carcinogenic intermediates of polyaromatic hydrocarbons. DDE is judged to be carcinogenic, having caused both benign and

malignant liver tumors in two strains of mice, and benign liver tumors in Syrian Golden hamsters. DDD has been found to produce carcinogenic responses in mice and rats, and has not been tested in hamsters. The positive results of these DDT metabolites are seen by the CAG as constituting limited additional evidence of the carcinogenicity of DDT (and possibly also of dicofol).

The observed lack of advanced states of malignancy, i.e., no extensive invasiveness and no metastasis, in any of the long-term positive bioassay studies is viewed as indicative of the limited carcinogenic potential of dicofol, DDT, DDE, or DDD. The absence of advanced malignant states in any of the positive rodent studies constitutes a diminution of the likelihood that these substances are carcinogenic in biotest animals.

In summary, the animal evidence for carcinogenicity of DDT is as follows:

Animal biotest species	Evidence for (+) or against (-) the carcinogenicity of DDT
mice	adequate (+)
rats	limited (+)
hamster	adequate negative evidence (-)
fish	limited (+)
dogs	inadequate
monkeys	limited negative evidence (-)

6.2. POSSIBLE CHEMICAL CARCINOGENICITY TO HUMANS AS A RESULT OF DDT EXPOSURE

The existing epidemiologic data base, because of its inadequacy, is not seen to contribute to the weight of evidence for the carcinogenicity of either DDT or dicofol.

6.3. OVERALL EVALUATION OF THE EVIDENCE FOR THE CARCINOGENICITY OF DDT

It has been customary within the EPA to assume that an overall carcinogenic response constitutes sufficient evidence for the carcinogenicity of a substance if two different biotest species respond in a sufficiently positive fashion.

This has not occurred in the case of DDT. While the above results show a wide species variability in oncogenic responses to dietary DDT residues, the results, taken as a whole, indicate a more positive than negative character in the test responses. The response in mice has clearly been positive, while rats have shown limited positive responses. Trout, although showing positive responses, provide an uncertain biotest for determining carcinogenicity to man. These results fall short of the two positive tests in animal species necessary for considering that sufficient evidence exists for the carcinogenicity of DDT. The negative result in hamsters is not an important factor in the present weight-of-evidence decision, since hamsters, unlike mice and humans, do not readily convert DDT to DDE. The results in dogs do not represent adequate evidence, and the negative results in monkeys, although important and interesting, were from studies whose duration was insufficient for a complete evaluation to be made. Taking into account the auxiliary information on positive genotoxicity and on the promotion character of DDT with a number of known carcinogens, the evidence for the carcinogenicity of DDT is judged to be equivalent to that representing positive biotest results in two animal species. This would place DDT in Group 2B of the IARC's classification system, which is equivalent to EPA's Group B2 (U.S. EPA, 1984), indicating that there is sufficient evidence in animals and inadequate data in humans as to the carcinogenicity of DDT. Agents in IARC's Group 2B (EPA's Group B2) are considered probably ($p > 0$) carcinogenic in humans. The lack of human data and the difficulties in relating test animal tumors to tumors in man preclude the exact quantitation of the likelihood that DDT is a human carcinogen.

7. SELECTED ANIMAL STUDIES TO ESTIMATE THE PUTATIVE CANCER POTENCIES OF DICOFOL, DDT, DDE, AND DDD - QUANTITATIVE DISCUSSION

7.1. JUSTIFICATION AND RISK METHODOLOGY

The weight of evidence for the carcinogenicity of DDT is assumed to mirror that of dicofol, DDE, and DDD, for which the data bases are less extensive. The evidential conclusions on the basis of animal studies are that DDT is definitely carcinogenic in one species (the mouse), is of limited carcinogenicity in two other species (rat and fish), and is not carcinogenic in hamsters (although DDE is carcinogenic to some extent in hamsters). Experimentally limited studies in dogs and monkeys suggest that DDT may have no carcinogenic effect in these species, but this has not been established. Epidemiologic studies have been inadequate to determine whether or not DDT has any carcinogenic effect in humans.

Additional positive mutagenicity data, especially positive translocation data and tumor initiation/promotion studies, in which DDT has been shown to promote the initiation effects of some carcinogens, have contributed to the positive evidence in one species and to the limited evidence in two other species. The result has been to increase the weight of evidence for the carcinogenicity of DDT so that it is equivalent to positive evidence in two animal species. DDT is consequently judged to belong in IARC's Group 2B (which is equivalent to EPA's Group B2).

The weight of evidence concerning DDT and dicofol indicates that they are probable ($p > 0$) human carcinogens. Under these circumstances, it is the CAG's policy to estimate the 95% upper confidence limit (UCL) of cancer potency from the appropriate animal studies. This is done with recognition of the uncertainties that unavoidably enter into such weight-of-evidence considerations, and

with the recognition that DDT and dicofol could, in fact, be human carcinogens. In such an instance, where a compound is assumed to be a human carcinogen, risk management employs the use of the 95% UCL of cancer potency to estimate a level of risk not likely to be exceeded under anticipated exposure conditions.

Only those studies that were well-conducted, showed significant increases in tumors in treated test animals, and showed a significant positive trend were chosen for the purposes of risk estimation. Generally, such factors as inadequate animal care, inadequate reporting, insufficient number of animals, etc., were criteria for rejection of a study. In retrospect, however, no rejected study would have significantly changed the CAG's overall estimation of cancer potency.

The CAG calculates cancer potency estimations by means of the linearized multistage model originally described by Crump et al. (1976, 1977). The finalized methodology for risk estimation using the multistage model was published in the Federal Register in 1980 (U.S. EPA, 1980b) and is recommended for use in the Proposed Guidelines for Carcinogen Risk Assessment (U.S. EPA, 1984). These methods also have been described in some detail by Dr. E. Anderson and the CAG (Anderson et al., 1983). The computer program used to estimate cancer potency in this document was written by Crump and his collaborators. The program, GLOBAL79, generated maximum likelihood estimates of the 95% UCL of cancer potency. The upper-bound limit of 95% was selected as a reasonable upper limit, but is not linked to a known biological truth of the actual cancer potency estimate. The cancer potency is estimated by the q_1^* term of the multistage model and has the unit $(mg/kg\ body\ weight/day)^{-1}$. This q_1^* indicates the 95% UCL of the slope at low exposure levels, and when multiplied by the best average lifetime exposure estimate ("d" in units of mg/kg/day), gives

an upper-bound estimate of the lifetime risk (unitless): upper bound risk $\cong q_1^* d$.

7.2. DICOFOL - MICE AND RATS

The main thrust of this document is to determine the carcinogenicity of dicofol and its contaminants, DDT, DDE, and DDD. Technical-grade dicofol (85%-90% active ingredient, according to the Office of Pesticide Programs) containing these contaminants, was tested by NCI in a 2-year bioassay in mice and rats (NCI, 1978a). Only B6C3F1 male mice responded with tumors; Osborne-Mendel rats in a parallel experiment did not (Table 4).

Considering the possibility that dicofol is, in fact, a human carcinogen, its quantitative cancer potency was estimated as shown in Table 4. It is notable that this response occurred as mostly malignant (> 97%), but non-metastasizing, tumors in B6C3F1 male mouse livers. The estimated cancer potency of technical-grade dicofol is as follows:

$$q_1^* = 0.44 \text{ (mg/kg/day)}^{-1}$$

7.3. DDT - MULTIGENERATION STUDIES - MICE

7.3.1. Hungarian Study - Institute for Nutrition, Budapest, Hungary

One of the first studies of DDT was a multigeneration study in which BALB/c mice were fed DDT continuously for their lifetimes (Tarjan and Kemeny, 1969). Five generations were each fed 3 ppm DDT, and each mouse was examined for tumors after a lifetime of ingesting DDT.

Unlike studies of dicofol (Section 7.2.) and other studies of DDT in mice (Sections 7.3. and 7.4.), this study did not produce a significant liver response: 3 benign hepatomas/683 mice, as compared to 0/406 in control BALB/c mice. Only lung tumors (41.3% of the observed tumors) and leukemias (30.2% of

TABLE 4. INCIDENCE OF HEPATOCELLULAR CARCINOMAS AND BENIGN LIVER TUMORS IN B6C3F1 MALE MICE FED DICOFOL[a]

Site/Dose group	Hepatomas	Hepatocellular carcinomas	Combined
Liver			
0 ppm[b]	0/18 (0)	3/18 (17)	3/18 (17) $p<0.001$[d]
264 ppm[c]	1/50 (2)	22/50 (44)	23/50 (46) $p=0.035$[e]
528 ppm[c]	1/47 (2)	35/47 (74)	36/47 (76) $p<0.001$[e]
q_1^*	ND	ND	0.44

[a]Number of animals with tumors/number of animals examined (percent). Only male mice responded; female B6C3F1 mice did not respond. Male B6C3F1 responses were mostly malignant liver tumors, but no metastases.
[b]Technical-grade dicofol (85%-90% active ingredient) was obtained from Rohm and Haas; OPP states that this is representative of present-day technical-grade dicofol.
[c]Human equivalent dose (mg/kg/day) = 0.006067 (ppm in mouse diet); 0.006067 = [0.13 mg/kg/ day x $(0.03/70)^{1/3}$ x 5 days/7 days/wk x 78 wk/90 wk average lifetime]. The factor 0.13 mg/kg/day comes from the correlation of ppm concentration in the mouse diet to an average daily rate of intake in units of mg/kg/day. The average test mouse is assumed to weigh 0.03 kg and man, 70 kg.
[d]Probability that there is a trend to this data set at a statistical level of "p".
[e]Probability that this incidence is significant compared to controls at a statistical level of "p".
ND = not done.

SOURCE: NCI, 1978a.

the observed tumors) are considered significant; the remaining tumors appeared not to be dose-related or in excess of those same tumors occurring in control mice.

Table 5 shows the tumor incidence of lung carcinomas and leukemias generation by generation. Generation F_1 does not have enough animals for the carcinogenic results to be interpreted, but lung tumors were significantly increased by F_2, and then through F_5; in addition, leukemias were increased by F_3, and then through F_5. The authors state that the historical rate of lung carcinomas in BALB/c mice is < 0.1 percent, and that spontaneous leukemias are unknown in BALB/c mice. Therefore, these increases in lung carcinomas and leukemias are significant when compared to external as well as internal negative controls.

The q_1^* is likely not to be statistically stable by F_2 in the case of the lungs, due to the small number of mice generated by the F_2 generation. The F_3, F_4, and F_5 generations are not dissimilar in cancer potency for both lung carcinomas and leukemias. The overall tumor results for all five generations are summarized at the bottom of Table 5.

These potency results are not dissimilar to those in Table 5 (top) F_3 - F_5, and further, lung carcinoma and leukemia potencies are also not dissimilar. Thus, an overall geometric collective average of F_3 - F_5 for lung and leukemia cancer potencies = 7.27 $(mg/kg/day)^{-1}$ (potency variation: 4.83 to 9.98). These results compare to the CAG's estimate of 8.42 $(mg/kg/day)^{-1}$ for lung tumors for DDT presented in the previous Water Quality Document on DDT (prepared by Drs. McGaughy and Singh of the CAG (U.S. EPA, 1980a).

The results of this 1969 study by Tarjan and Kemeny are clearly different in organ site (lung/leukemia versus liver) and cancer potency (about an order of magnitude greater) from most of the other studies reviewed in Table 1.

TABLE 5. INCIDENCE OF THE MOST COMMONLY OCCURRING MALIGNANT TUMORS IN EACH OF FIVE GENERATIONS OF BALB/c MICE FED DDT

Site/Dose group	Incidence by generation[a] (combined male and female)(%)				
	F_1	F_2	F_3	F_4	F_5
Lung (carcinomas)					
Control	0/3(00.0)	0/39(00.0)	3/51(5.9)	0/144(00.0)	2/169(1.2)
3 ppm DDT[b]	2/10(20.0)	10/35(28.5)	13/69(18.8)	41/264(15.5)	50/305(16.4)
Significance[c]	--	p=0.001	p=0.007	p<0.002	p<0.001
q_1^*[d]	--	18.78	9.09	7.45	7.37
q_1^*[e]	--	17.20	9.95	7.16	7.68
Leukemia					
Control	2/3(66.6)	1/39(2.6)	0/51(00.0)	3/144(2.1)	4/169(2.4)
3 ppm DDT[b]	4/10(40.0)	2/35(5.7)	11/69(15.9)	35/264(13.2)	33/305(10.8)
Significance[c]	--	p=0.924	p=0.008	p<0.001	p=0.002
q_1^*[d]	--	4.67	9.48	5.79	4.50
q_1^*[e]	--	5.01	8.98	6.22	4.83

[a]Number of animals with tumors/number of animals examined (percent). The F_1 generation contained too few effective animals for reliable statistical analysis.
[b]The human equivalent doses are calculated by multiplying the ppm values by 0.13 and then by the cube root of 0.030/70. 3 ppm DDT to BALB/c mice = 0.029 mg/kg body weight/day for humans. The DDT was given to the mice for lifetime via the diet every day, so no time correction is necessary.
[c]Beneath each dose group incidence is the p value for the comparison of the dose group incidence with that of the control group. The F_1 generation was not analyzed.
[d]The q_1^*'s were calculated using the human equivalent dose. The index values assume that DDT contamination in the control diets was zero.
[e]The q_1^*'s were calculated using a level of 0.20 ppm DDTr (combined DDT-related residues) in the control feed, as reported by the authors.

SUMMARY OF THE ABOVE TUMOR INCIDENCE
(combined males plus females for all five generations)

Site/DDT dose group (ppm)		Combined incidence	Resulting cancer potency q_1^* $(mg/kg/day)^{-1}$
Liver (benign)	0	0/406 (0)	
	3	3/683 (0.44%)	not calculated
Leukemia	0	10/406 (2.5%)	
	3	85/683 (12.4%)	4.68
Lung (carcinomas)	0	5/406 (1.2%)	
	3	116/683 (17.0%)	7.06

SOURCE: Tarjan and Kemeny, 1969.

7.3.2. French Study - IARC, Lyon, France

A six-generation study in CF-1 mice has been reported in which DDT was incorporated in the diet at 0, 2, 10, 50, and 250 ppm (Turusov et al., 1973). Table 6 shows the benign liver tumor results (referred to as hepatomas) by generation for each of six generations. Historical control incidences for hepatomas in CF-1 mouse livers have been found to be 20 percent in males and 13 percent in females. The liver response appears to be an increase in an already-present event in untreated CF-1 mice controls. There is no statistical trend in the q_1^* values with the successive generations, which indicates that there is no buildup of cancerous effects passed vertically from generation to generation. The CAG therefore views each generation as an independent trial, and has calculated geometric averages to express the central tendency of the data. Thus, the geometric averages of the q_1^* values are 0.80 $(mg/kg/day)^{-1}$ (males) and 0.42 $(mg/kg/day^{-1})$ (females), with variation from 0.37 to 1.10 $(mg/kg/day)^{-1}$.

7.3.3. Italian Study - National Institute for the Study and Cure of Cancer, Milan, Italy

A two-generation study in BALB/C mice was performed in which 0, 2, 20, and 250 ppm DDT was incorporated into the diet (Terracini et al., 1973). Mice were fed DDT continuously for a lifetime. The results (Table 7) indicated only benign liver tumors. While these tumors were benign in appearance, they had a malignant characteristic in that they were transplantable in syngenetic mice. No metastases were observed. Essentially, doses at 20 ppm and below were inactive in producing liver tumors, whereas at the next highest dose, 250 ppm, liver tumors became abundant. At the highest dose tested, 250 ppm, there were body weight losses and decreased survivals due to toxicity.

The total number of tumor-bearing BALB/c mice did not vary among treatment groups and controls, thereby indicating only a change in tumor pattern at

TABLE 6. INCIDENCE OF BENIGN LIVER TUMORS IN EACH OF SIX GENERATIONS OF CF-1 MICE FED DDT

Sex/Dose group	Benign liver tumor incidence by generation (%)[a]					
	Parental	F_1	F_2	F_3	F_4	F_5
Males						
Control	14/60(24)	13/60(21)	20/60(34)	21/60(35)	16/60(26)	23/60(39)
2 ppm[b]	26/60(44)	29/60(48)	38/60(63)	30/60(50)	34/60(57)	25/60(42)
10 ppm	32/60(53)	28/60(47)	33/60(55)	36/60(60)	24/60(40)	26/60(44)
50 ppm	27/60(45)	35/60(58)	41/60(69)	36/60(60)	32/60(53)	28/60(47)
250 ppm	46/60(76)	51/60(85)	53/60(89)	53/60(89)	57/60(95)	48/60(80)
q_1^{*}[c]	0.572	0.873	0.935	0.878	1.096	0.598
Females						
Control	3/60(5)	2/60(3)	1/60(2)	2/60(3)	4/60(7)	5/60(8)
2 ppm[b]	3/60(5)	1/60(2)	3/60(5)	5/60(9)	0/60(0)	0/60(0)
10 ppm	2/60(3)	8/60(13)	8/60(13)	3/60(5)	5/60(8)	6/60(10)
50 ppm	8/60(13)	7/60(12)	8/60(13)	9/60(15)	10/60(16)	7/60(11)
250 ppm	37/60(61)	43/60(71)	31/60(52)	40/60(67)	48/60(80)	38/60(64)
q_1^{*}	0.372	0.471	0.369	0.434	0.526	0.370

[a]Number of animals with tumors/number of animals examined (percent). The effective number of animals was given by Turusov et al. as 50-60; 60 has been used for every group because the exact number was not given.

[b]The human equivalent doses are calculated by multiplying the ppm values by 0.13 and then by the cube root of 0.030/70 (=0.0753949). No adjustment for time was made because these were lifetime tests and CF-1 mice were fed DDT continuously during that time. For example, human equivalent doses are: 2 ppm=0.0196, 10 ppm=0.0980, 50 ppm=0.4900, and 250 ppm=2.45 mg/kg body weight/day.

[c]The q_1^{*}'s of the upper-bound limits in units of (mg/kg body weight/day of dietary exposure)$^{-1}$ were calculated using the multistage model as described in section 7.1).

SOURCE: Turusov et al., 1973.

TABLE 7. INCIDENCE OF BENIGN LIVER TUMORS IN BALB/c MICE FED DDT DURING A TWO-GENERATION EXPERIMENT[a]

Dose group	Incidence of benign liver tumors by generation[b]		
	Males	Females	
	Parental + F_1	Parental	F_1
0 ppm Trend[c]	2/107(1.9) p<0.001	0/62(0) p<0.001	0/69(0) p<0.001
2 ppm	3/112(2.7)	0/63(0)	0/73(0)
20 ppm	1/106(0.9)	1/61(1.6)	0/67(0)
250 ppm	15/106(14.2)	28/63(44.4)	43/58(74.1)
q_1^{*}[d]	0.074	0.080	0.094
High dose q_1^{*}	0.086	0.324	0.718

[a]Number of animals with tumors/number of animals examined (percent). Malignant tumors were not observed in liver.

[b]The numbers in the groups of males were reduced by fighting, so the two generations of males were pooled. Each high-dose group shown is statistically different from its control group (p<0.001). Other pairwise tests were not significant.

[c]Beneath the control incidence is the p value for positive trend in incidence over the dose levels.

[d]The q_1^{*}'s were calculated using the human equivalent dose. The "high-dose q_1^{*} is the result of using only the controls and the high-dose groups in the calculations. The human equivalent doses are calculated by multiplying the ppm values by 0.13 and then by the cube root of 0.030/70 (= 0.0753949). For example, 250 ppm = 2.45 mg/kg/day for humans.

SOURCE: Terracini et al., 1973.

the highest dose tested, 250 ppm. Assuming that this study is predictive to humans, and for the sake of comparison to the other multigeneration studies in mice (Sections 7.3.1. and 7.3.2.), the q_1^* was calculated (Table 7). The values are similar between parental and F_1 generations and between males and females. Thus, a collective q_1^* was calculated. The geometric average was q_1^* = 0.082 $(mg/kg/day)^{-1}$, with a range of variation of 0.074 - 0.094 $(mg/kg/day)^{-1}$.

7.4. DDT - SINGLE-GENERATION STUDIES - MICE

7.4.1. English Study - Shell Research Ltd., Kent, England

CF-1 mice were fed 0 and 100 ppm DDT continuously (in the feed) for a lifetime (110 weeks) (Thorpe and Walker, 1973). Survivals were good in this experiment and no overt toxicity from DDT was observed at 100 ppm; however, liver enlargement was observed as early as 50 weeks. The tumor results are given in Table 8. Both benign and malignant liver tumors were increased significantly in the liver of CF-1 mice, but the total tumor-bearing CF-1 mice did not differ among controls and treated groups. The q_1^* values were calculated for males [0.52 $(mg/kg/day)^{-1}$] and for females [0.81 $(mg/kg/day)^{-1}$] (Table 8).

7.4.2. U.S.A. Study - NCI, Bethesda, Maryland

B6C3F1 mice were fed DDT at 0, 22, and 44 ppm (males) and 87 and 175 ppm (females) for 78 weeks of continuous dosing followed by 15 weeks of observation before terminal sacrifice (NCI, 1978b). No evidence for carcinogenicity was observed in this study.

7.4.3. Italian Limited-Exposure Study - National Institute for the Study and the Cure of Cancer, Milan, Italy

In another study, CF-1 mice were fed 0 or 250 ppm DDT for 15 or 30 weeks and then observed for 65, 95, or 120 weeks before sacrifice (Tomatis and Turusov, 1975). Table 9 gives the incidence of benign liver tumors. No other tumor types were significantly increased. Increased time of exposure to 250 ppm DDT

TABLE 8. INCIDENCE OF LIVER TUMORS (BENIGN AND MALIGNANT) IN CF-1 MICE FED DDT FOR A SINGLE GENERATION

Dose group	Incidence of benign liver tumors[a]	Incidence of malignant liver tumors[a]
Males		
Controls	11/45 (24%)	2/45 (4.4%)
100 ppm	23/30 (80%)	9/30 (30%)
q_1^*	ND	0.52
Females		
Controls	10/44 (23%)	0/44 (0%)
100 ppm	26/30 (87%)	12/30 (40%)
q_1^*	ND	0.81

[a]Benign liver tumors in this study were referred to as "type a" and malignant liver tumors as "type b."
ND = Not determined.

SOURCE: Thorpe and Walker, 1973.

TABLE 9. INCIDENCE OF BENIGN LIVER TUMORS IN CF-1 MICE FED DDT FOR 15 OR 30 WEEKS AND THEN SACRIFICED AT 65, 95, AND 120 WEEKS[ab]

Dose group	Males at week--			Females at week--		
	65	95	120	65	95	120
0 ppm	12/70(17)	24/83(29)	33/98(34)	0/69(0)	0/72(0)	1/90(1)
250 ppm for 15 weeks[d]	13/60(22) p=0.142	25/60(42) p=0.040	25/60(42) p=0.080	3/60(5) p=0.097	11/60(14) p<0.001	5/50(10) p=0.034
250 ppm for 30 weeks[d]	38/60(63) p<0.001	41/60(68) p<0.001	37/60(62) p<0.001	4/54(7) p=0.034	11/55(20) p<0.001	11/54(20) p<0.001
q_1^{*e} values						
all q_1^*	0.36	1.04	0.84	0.19	0.49	0.35
30 week	1.38	1.43	1.06	0.19	0.79	0.43

[a]Number of animals with tumors/number of animals examined (percent).
[b]Some groups were exposed for 15 weeks; other groups were exposed for 30 weeks. All groups were sacrificed serially at 30, 65, 95, and 120 weeks.
[c]The human equivalent dose for 1 ppm for 15 weeks is 0.4084 mg/kg/day and for 30 weeks is 0.8168 mg/kg/day. The human equivalent doses are calculated by multiplying the ppm values by 0.13 and then by the cube root of 0.030/70 (= 0.0753949). Adjustments for time consist of multiplying the 15-week dose by 15/90 and the 30-week exposure by 30/90.
[d]Beneath each dosed group incidence is the p value for comparison of the incidence in the dose group with that in the control group.
[e]The q_1^*'s were calculated based on the human equivalent dose shown in footnote c. The term "all q_1^*" indicates that the dosed groups and the control group were used in the calculation. The "30 week" row contains the results of using only the 30-week exposure cancer data with the control cancer data.

SOURCES: Tomatis and Turusov, 1975; Tomatis et al., 1974a.

was proportional to increased total dose of DDT, which in turn appears to be functionally linked to increased benign liver tumors in both males and females. The appearance of benign liver tumors was observed earlier than in other studies using this strain. These liver tumors increased in size with longer exposure to DDT. Thus, the latency period for benign liver tumors in CF-1 mice was decreased in the 250-ppm dose group. Removal of CF-1 mice from DDT exposure did not cause tumor regression in the liver; instead, the DDT-induced benign liver tumors continued to grow. Such a continuance of growth, even in the absence of DDT, suggests autonomous growth, a malignant characteristic. The response in males was manifest by 65 weeks in the 250-ppm group, which was dosed for 30 weeks ($p < 0.001$, Table 9) and in females was manifest by 65 to 95 weeks. The response was greatest in the males, but male controls also had benign liver tumors as early as 65 weeks (12/70, 17%), whereas female controls at 65, 95, or 120 weeks were devoid of benign liver tumors. The male liver response is apparently a stimulation of a process occurring in controls, in contradistinction to the female liver response, which is the de novo formation of tumors with exposure to DDT.

The comparable cancer potency, q_1^*, to other studies reviewed in this document is at 95 weeks, approximately equivalent to the lifetime of a mouse, and the usual time of termination in other studies reviewed in this document. A dosage rate of 250 ppm for 15 weeks is one-half of the total dose of 250 ppm for 30 weeks, and thus, the two dose times of 15 and 30 weeks will be treated as different graded dose groups, with the resulting upper-bound limit of cancer potencies being as follows:

$$\text{Males:} \quad q_1^* = 1.04 \text{ (mg/kg/day)}^{-1}$$

$$\text{Females:} \quad q_1^* = 0.49 \text{ (mg/kg/day)}^{-1}$$

7.5. DDT - SINGLE-GENERATION STUDIES - RATS

7.5.1. U.S.A. Study - Eppley Institute for Research in Cancer, Omaha, Nebraska

MRC Portion rats (Wistar-derived) were fed 0, 125, 250, or 500 ppm DDT for essentially the natural lifetime of this strain of rat (Cabral et al., 1982b). The total number of tumor-bearing rats did not vary with dosage. The female rats responded with a slight increase in benign liver tumors, which were neither invasive locally nor disseminated to other organs (Table 10). The male rats did not respond. The tumor response in female rats was weak compared to the response in mice (Section 7.4.). The upper-bound limit of cancer potency for the female MRC Portion rat is estimated to be:

$$q_1^* = 0.084 \text{ (mg/kg/day)}^{-1}$$

7.5.2. Italian Study - Institute of Oncology, Genoa, Italy

Wistar strain rats were fed 0 or 500 ppm DDT in the diet for their lifetimes (Rossi et al., 1977). The total number of tumor-bearing animals (TBA) increased to some degree; male TBA controls, 19/35 (54.3%), increased to 19/27 (70.4%) in the 500-ppm DDT group, whereas female TBA controls, 19/32 (59.4%), increased to 23/28 (82.1%). Such increases in tumor-bearing animals are considered moderate.

The incidence of benign liver tumors was increased ($p < 0.001$) at the rather high dose of 500 ppm DDT (Rossi et al., 1977) (Table 10). Liver tumors that were similar in appearance and incidence were observed in rats treated with phenobarbital, thereby suggesting that DDT, like phenobarbital, is a liver tumor promoter. The upper-bound limits of the cancer potency for DDT are estimated to be as follows:

TABLE 10. INCIDENCE OF BENIGN LIVER TUMORS IN RATS FED DDT[a]

Dose group[d]	Cabral et al.[b]		Rossi et al.[c]	
	Males	Females	Males	Females
0 ppm	1/38(0)	0/38(0)	0/35(0)	0/32(0)
Trend[e]	NS	p=0.003	--	--
125 ppm	0.30(0)	2/30(6.7)	--	--
	NS	NS		
250 ppm	1/30(3.3)	4/30(13.3)	--	--
	NS	p=0.033		
500 ppm	2/38(5.3)	7/38(18.4)	9/27(33.3)	15/28(53.6)
	NS	p=0.005	p<0.001	p<0.001
q_1^{*}[f]	ND[g]	0.084	0.16	0.27

[a]Number of animals with tumor/number of animals examined (percent).
[b]These were Portion (Wistar derived) rats.
[c]These were Wistar rats.
[d]The human equivalent doses are calculated by multiplying the ppm values by 0.0085499, which is 0.05 mg/kg/day (for rats) multiplied by the cube root of 0.350/70 (=0.0753949). No adjustment for time was made because rats were fed continously for a lifetime.
[e]Beneath the control group incidence is the p value for a positive trend of incidences as the dose increases, when the p value is less than p=0.05, otherwise NS (not significant). Beneath each dosed group incidence is the p value for the comparison of the incidence in the dosed group with its control group when it is less than p=0.05, otherwise NS.
[f]The q_1^{*}'s were calculated using the human equivalent dose. For example, 500 ppm = 4.275 mg/kg/day for humans.
[g]Not calculated due to lack of statistical increase in hepatomas.

NS = Not significant.
ND = Not determined.

SOURCES: Cabral et al., 1982b; Rossi et al., 1977.

Males: $q_1^* = 0.16\ (mg/kg/day)^{-1}$

Females: $q_1^* = 0.27\ (mg/kg/day)^{-1}$

7.6. DDT - SINGLE-GENERATION STUDIES - HAMSTERS

7.6.1. U.S.A. Study - National Institutes of Health, Bethesda, Maryland

Syrian Golden hamsters were fed DDT at levels of 0, 125, and 500 ppm (Cabral et al., 1982a). The number of tumor-bearing animals in male Portion-Wistar rats did not vary with dosage of DDT. Male hamsters did not exhibit liver tumors, but mice and rats did exhibit liver tumors at comparable levels of DDT (Cabral et al., 1982a) (Table 11).

Female hamsters showed a mild trend in the total tumor-bearing animals ($p < 0.05$):

Dose	Total tumor-bearing female hamsters
Control	3/40 (7.5%)
125 ppm	5/30 (16.6%)
250 ppm	8/31 (25.8%)
500 ppm	11/39 (28.2%)

Female hamsters, however, did not show a liver tumor response (Cabral et al., 1982a) (Table 10).

Responses were marginal or nonexistent in male and female hamster adrenal glands (Table 12). The male hamster adrenal response is not considered statistically significant, nor is that of the female, which did not differ from controls, even though there is a trend of $p = 0.022$. All other tumors appeared random in occurrence in both male and female hamsters.

The Cabral et al. (1982a) study indicated a lack of DDT activity in hamsters.

TABLE 11. INCIDENCE OF BENIGN LIVER CELL TUMORS IN HAMSTERS FED DDT OR DDE[a]

Dose Group[b]	Cabral et al. (1982a) DDT		Rossi et al. (1977) DDT		Rossi et al. (1983) DDE	
	Males	Females	Males	Females	Males	Females
Controls Trend[c]	0/40(0) NS	0/39(0) NS	0/10(0) NS	0/31(0) NS	0/10(0) NS	0/31(0) p=0.011
125 ppm	0/30(0) NS	0/28(0) NS	--	--	--	--
250 ppm	3/31(10) NS	0/28(0) NS	--	--	--	--
500 ppm	0/39(0) NS	0/40(0) NS	--	--	7/15(46) p=0.013	4/26(15) p=0.037
1000 ppm	--	--	0/17(0) NS	0/26(0) NS	8/24(33) p=0.040	5/24(21) p=0.012
q_1^{*}[d]	ND[e]	ND	ND	ND	0.093	0.046

[a]Number of animals with tumors/number of animals examined (percent).
[b]The human equivalent doses are calculated by multiplying ppm by 0.08 and by the cube root of 0.120/70 = 0.119682. For example, 1000 ppm = 9.57 mg/kg/day for humans.
[c]Beneath the control group incidence is the p value for positive trend over increased dose and beneath the dosed group incidences is the p value for increased incidence in that group when compared with the controls. If the value is larger than p=0.05 then NS is entered.
[d]The q_1^{*}'s were calculated based on the human equivalent doses.
[e]Due to lack of statistical increase in tumors, q_1^{*} was not determined.
NS = Not significant.
ND = Not determined.

SOURCE: Cabral et al., 1982a; Rossi et al., 1977, 1983.

TABLE 12. INCIDENCE OF ADENOMAS IN THE ADRENAL IN SYRIAN GOLDEN HAMSTERS RECEIVING DDT[a]

Dose group[b]	Cabral et al.		Rossi et al.	
	Males	Females	Males	Females
0 ppm	3/40(8)	0/39(0)	8/31(26)	2/42(5)
Trend[c]	NS	p=0.022	--[d]	--[d]
125 ppm	4/30(13) NS	0/28(0) NS	--[e]	--[e]
250 ppm	6/31(19) NS	1/28(3) NS	--[e]	--[e]
500 ppm	8/39(20) NS	3/40(8) NS	--[e]	--[e]
1000 ppm	--[e]	--[e]	14/35(40) NS	10/36(28) P=0.005
q_1^*	--[f]	--[f]	--	0.051

[a]Number of animals with tumors/number of animals examined (percent).
[b]The human equivalent doses are calculated as ppm x 0.0095746, which is 0.08 multiplied by the cube root of 0.120/70(=0.119862). For example, 1000 ppm = 9.57 mg/kg/day human equivalent dose.
[c]Beneath the control group incidence is the p value for positive trend as doses increase. Beneath each dosed group incidence is the p value for a significant increase in incidence in that dosed group compared with the control group incidence. When the p value is greater than p=0.05, NS (not significant) is used.
[d]It is not possible to determine a valid trend with only one control and one dose group.
[e]Hamsters were not dosed at this level in this experiment.
[f]Since the dosed groups are not significantly increased over controls, neither the calculated female q_1^* = 0.038 nor the male q_1^* = 0.039 is considered relevant.

NS = Not significant.

SOURCE: Cabral et al., 1982a; Rossi et al., 1983.

7.6.2. Italian Study - Scientific Institute for the Study and Cure of Cancer, Genoa, Italy

In this study, Syrian Golden hamsters were dosed with 0 or 1000 ppm DDT (Rossi et al., 1983). The tumor-bearing animals (TBA) did not vary with DDT dosage, and there were no dose-related increases in any specific tumor type, including liver tumors. Rossi did observe an adrenal response (Table 12), where the response gave rise to a q_1^* of 0.051 $(mg/kg/day)^{-1}$.

7.7. DDE AND DDD SINGLE-GENERATION STUDIES

7.7.1. Italian Study - Institute for the Study and Cure of Cancer, Genoa, Italy (Rossi et al., 1983)

In the same Italian study as cited in Section 7.6.2. above, DDE was fed in doses of 0, 500, and 1000 ppm to hamsters (Table 11). As with DDT, the TBA did not vary with DDE dosage. However, a carcinogenic response in the liver was observed in the form of neoplastic nodules. The number of nodules/hamster (multiplicity = 2 to 5) increased with dose, as did the size of the liver nodules (diameter variation = 4 to 10 mm). These incidences (Rossi et al., 1983) (Table 11) indicate marginal, but real, hamster liver carcinogenicity of DDE, a DDT metabolite.

Thus, the upper-bound limits of cancer potency for DDE in hamsters are estimated to be as follows:

$$\text{Males:} \quad q_1^* = 0.093 \ (mg/kg/day)^{-1}$$

$$\text{Females:} \quad q_1^* = 0.046 \ (mg/kg/day)^{-1}$$

7.7.2. U.S.A. Study, National Institutes of Health, Bethesda, Maryland

In an NCI study of Osborne-Mendel rats (1978b), DDE doses of up to 839 ppm in the diet did not induce carcinomas. In the same study, DDE doses of 0, 148,

and 261 ppm in the diet of B6C3F1 mice were given for 78 weeks, and the surviving mice were observed for 15 weeks more before termination. The tumor response in B6C3F1 mice is shown in Table 13.

There were clear increases both in total tumor-bearing animals (both sexes) and in a specific tumor type, namely, liver hepatocarcinomas (both sexes). Table 13 shows both a significant trend and increases in the hepatocellular carcinomas at 148 and 261 ppm DDE as compared to controls.

The DDE cancer potencies in mice are estimated to be as follows:

$$\text{Males:} \quad q_1^* = 0.34 \ (\text{mg/kg/day})^{-1}$$

$$\text{Females:} \quad q_1^* = 0.82 \ (\text{mg/kg/day})^{-1}$$

7.7.3. Italian Study - National Institute for the Study and the Cure of Cancer, Milan, Italy (Tomatis et al., 1984)

This study was designed to test the carcinogenic responses of DDE or DDD or a combination of the two fed to CF-1 mice for a lifetime (Tomatis et al., 1974b). Dosages in the feed were 0, 250 ppm DDE, 250 ppm DDD, or 125 ppm DDE + 125 ppm DDD. Exposure to DDE caused higher incidences of mice dying early with hepatomas than did exposure to DDD, with the DDE + DDD group falling in the intermediate range. The numbers of tumor-bearing animals (TBA) of both sexes did not vary significantly from controls at terminal sacrifice. However, benign liver tumors were increased in a dose-related manner in all three groups, DDE, DDD, and DDE + DDD (Table 14). DDE seems to be somewhat more potent than DDD in causing benign tumors. The resulting upper-bound limits of cancer potency are estimated in Table 14.

TABLE 13. INCIDENCE OF HEPATOCELLULAR CARCINOMAS IN B6C3F1 MICE FED DDE[a]

Dose group[b]	Tumor-bearing animals with malignant tumors	Number of animals with hepatocellular carcinomas
Males		
0 ppm	0 (0)	0/19 (0) p<0.001[c]
148 ppm	13/41 (31.7)	7/41 (17) NS
261 ppm	22/47 (46.8)	17/47 (36) p<0.001
Cancer potency[d]		$q_1^* = 0.34$
Females		
0 ppm	2/19 (10.5)	0/19 (0) p<0.001
148 ppm	24/47 (51.1)	19/47 (40) p<0.001
261 ppm	35/48 (72.9)	34/48 (71) p<0.001
Cancer potency[d]		$q_1^* = 0.82$

[a]Number of animals with tumors/number of animals examined (percent).
[b]The human equivalent doses are calculated by multiplying the ppm by 0.006067, which is 0.13 x (cube root of 0.030/70) x 5/7 x 78/90. The 5/7 value represents 5 days a week of dosing, and 78/90 is intended to adjust for 78 weeks of exposure rather than a lifetime. For example, 148 ppm = 0.8979 mg/kg/day and 261 ppm = 1.584 mg/kg/day for humans.
[c]Beneath the control incidence is the p value for trend, and beneath each dosed group incidence is the p value for the comparison of that incidence with the control incidence.
[d]The q_1^* values were calculated using both of the dosed groups and the control group from each compound.

SOURCE: NCI, 1978b.

TABLE 14. INCIDENCE OF BENIGN LIVER TUMORS IN MICE RECEIVING DDE (WITH OR WITHOUT DDD)[a]

Dose group[b]	Benign liver tumors	
	Males	Females
0 ppm	33/98(34)	1/90(1)
Trend[c]		
250 ppm DDE	39/53(74)	54/55(98)
	p<0.001	p<0.001
250 ppm DDD	31/59(52)	1/59(2)
	p=0.009	NS
125 ppm DDE+ 125 ppm DDD	42/56(75)	42/55(76)
	p<0.001	p<0.001
q_1^{*}[d]		
DDE alone	0.553	2.544
DDD alone	0.248	--[e]
DDE + DDD	0.576	0.765

[a]Number of animals with tumors/number of animals examined (percent).
[b]The human equivalent doses are calculated by multiplying the ppm values by 0.13 and then by the cube root of 0.030/70 (= 0.0753949). No time correction was necessary.
[c]Beneath each dose group incidence is the p value for a positive increase in incidence in the dosed group when compared with its control group incidence. If the p value is greater than p=0.05, NS is entered.
[d]The q_1^{*}'s were calculated based on the human equivalent doses, e.g., 250 ppm = 245 mg/kg/day.
[e]Not calculated.

NS = Not significant.

SOURCE: Tomatis et al., 1974b.

7.8. SUMMARY OF QUANTITATIVE CANCER POTENCY ESTIMATION

Table 15 summarizes the studies modeled for low-dose risk extrapolation. Variability in q_1^* estimation can be seen in the following factors:

(1) differences from study to study in the same species and strains,

(2) differences among species,

(3) degree of malignancy, and

(4) differences in sex, with no discernible trend toward either sex

Notwithstanding the variability in q_1^* estimation, the DDT data fall into a range of q_1^* values of 0.082 to 7.27 $(mg/kg/day)^{-1}$, an 88-fold difference. It is judged to be likely that the Tarjan and Kemeny (1969) study provides an outlier value of the q_1^*. The Dixon statistical criterion for rejecting outlier values was applied, and the Tarjan and Kemeny study value was rejected from the remaining body of DDT carcinogenic potency data in Table 15 at the 0.01 level of probability (Natrella, 1966). This judgment is also based on the fact that the Tarjan and Kemeny bioassay is an old study, from an unaudited laboratory, using feed that was contaminated with DDT. It accounts for tumors in the lung and leukemias with no excess liver tumors, which is different from the organ site (liver) of the other six DDT studies selected for q_1^* estimation in Table 15.

Rejecting the Tarjan and Kemeny DDT study readjusts the range for q_1^* values to 0.082 to 1.04, a 13-fold difference, which is close to the order-of-magnitude difference that might be expected for inter-study variability. Within the 0.082 to 1.04 $(mg/kg/day)^{-1}$ range, no further refinement or rejection can be logically made, and thus a geometric average of these values (Table 15) is viewed as the best rational estimate of the upper-bound limit of the unit

TABLE 15. SUMMARY OF QUANTITATIVE CANCER POTENCY ESTIMATION FOR SELECTED POSITIVE BIOASSAYS FOR CARCINOGENICITY OF DICOFOL, DDT, DDE, AND DDD

Chemical	Study name (section of this document)	Positive surrogate test animal	Carcinogenic response				Multistage cancer potency		
			Sex	Tumor site	State of malignancy	Metastasis	q_1^* $(mg/kg/day)^{-1}$ Males	q_1^* $(mg/kg/day)^{-1}$ Females	q_1^* range $(mg/kg/day)^{-1}$
Dicofol	NCI (7.2)	B6C3F1 mice	M	Liver	Benign & malignant	None	0.44	--[a]	0 - 0.44
DDT	Tarjan (7.3.1)	BALB/C mice	M+F	Lung/leukemia	Malignant	None	7.27[b] (sexes combined)		4.83 - 9.98
DDT	Turusov (7.3.2)	CF-1 mice	M/F	Liver	Benign[c]	None	0.80[b]	0.42[b]	0.37 - 1.096
DDT	Terracini (7.3.3)	BALB/C mice	M+F	Liver	Benign (& malignant?)[d]	None	0.082[b] (sexes combined)		0.074 - 0.094
DDT	Thorpe (7.4.1)	CF-1 mice	M/F	Liver	Benign & malignant	None	0.52	0.81	--
DDT	Tomatis (7.4.3)	CF-1 mice	M/F	Liver	Benign (& malignant?)[d]	None	1.04	0.49	--
DDT	Cabral (7.5.1)	MRC portion rats	F	Liver	Benign only	None		0.084	--
DDT	Rossi (7.5.2)	Wistar rats	M/F	Liver	Benign only	None	0.16	0.27	-- --
DDE	Rossi (7.7.1)	Syrian Golden hamsters	M/F	Liver	Benign	None	0.093	0.046	--
DDE	NCI (7.7.2)	B6C3F1 mice	M/F	Liver	Malignant	None	0.34	0.82	--
DDE	Tomatis (7.7.3)	CF-1 mice	M/F	Liver	Benign	None	0.55	2.54	--
DDD[e]	Tomatis (7.7.3)	CF-1 mice	M	Liver	Benign	None	0.25	--[a]	--

[a]Did not respond with tumors, so no cancer potency was calculated.
[b]A geometric mean was taken of the individual generation q_1^* values in the multigeneration cancer bioassay.
[c]A few malignant tumors were observed, but most of the responses were in the form of benign tumors.
[d]It was not certain, due to reporting and/or pathological uncertainty in the degree of malignancy, whether there were malignant cells present, but possible malignant neoplasms were indicated.
[e]DDD is also known as TDE.

risk. Hence,

q_1^* geometric average) =

$(0.80 \times 0.42 \times 0.082 \times 0.52 \times 0.81 \times 1.04 \times 0.49 \times 0.084 \times 0.16 \times 0.27)^{1/10}$

$q_1^* = 0.34$ (mg DDT/kg of human body weight/day of dietary exposure)$^{-1}$

This q_1^* is different from the previous q_1^* estimation of 8.42 (mg/kg/day)$^{-1}$ using the Tarjan and Kemeny (1969) study. The above geometric average q_1^* for the mouse and rat carcinogenic response of 0.34 is 24-fold less than the previous estimate.

Interestingly, the geometric average of the DDE q_1^* data is the same as for DDT.

q_1^* (geometric average) =

$(0.093 \times 0.046 \times 0.34 \times 0.82 \times 0.55 \times 2.54)^{1/6}$

$q_1^* = 0.34$ (mg DDE/kg of human body weight/day of dietary exposure)$^{-1}$

The singular value for DDD in Table 15 is $q_1^* = 0.25$ (mg/kg/day)$^{-1}$. The CAG does not view this difference (0.25 versus 0.34) as significant given the errors inherent in cancer potency estimation.

Lastly, the cancer potency of dicofol is compared to the potencies of DDT, DDE, and DDD as follows:

	Dicofol	DDT	DDE	DDD
Estimated q_1^*:	0.44	0.34	0.34	0.25
Range of values:	no range	0.084-1.04	0.046-2.54	no range
Number of studies used to estimate the average q_1^* value:	1	6	3	1

The differences between 0.44 (dicofol) and any of the other values are considered not significant. Furthermore, more statistical weight can be placed on DDT since more studies were done on DDT as compared to dicofol. The overall weighted average of the cancer potencies of the four compounds is calculated to be 0.34. The rather close potency values of the four compounds suggest that all of these compounds, if carcinogenic to man, either have essentially the same cancer potency, or that they have a metabolite or an impurity common to all, which induces the liver carcinogenesis. It is judged, then, that the upper confidence limit of the cancer potencies for dicofol, DDT, DDE, and DDD can all be represented by the single value of 0.34 $(mg/kg/day)^{-1}$.

7.9. EXAMPLE RISK ESTIMATION

The recommended upper confidence limit of the cancer potency, 0.34 $(mg/kg/day)^{-1}$, can be used to estimate the upper confidence limit of risk expected for an anticipated average dietary exposure to humans. This assumes that DDT or dicofol causes human cancer, although such is not known, in fact, to be true at the present time. For example, if it is assumed that the average exposure, via the diet, is a combination of DDT, DDT metabolites, or dicofol adding up to 0.2 μg/day, then the equivalent exposure in mg/kg/day, for a 70-kg person, converts to 2.86×10^{-6} mg/kg/day. This exposure correlates to an upper confidence limit of risk of 0.971×10^{-6} [= 0.34 $(mg/kg/day)^{-1}$ x 2.86×10^{-6} mg/kg/day], or approximately one in a million chances of getting cancer.

Realistic estimates of exposure to DDT or dicofol in the human diet can be used to estimate upper confidence limits of expected cancer risks. Exposures have been taken from a recent DDT residue review (Spinder, 1983) in which average exposures at the maximum DDT usage in 1965 have been estimated in the past to be as high as 5.7×10^{-4} mg/kg/day for a 70-kg individual, whereas a more recent estimate in 1978 is 0.11×10^{-4} mg/kg/day. The later exposure follows

after the 1972 cancellation of DDT in the United States. These average exposure estimates correspond to upper-limit lifetime risks of 1.9×10^{-4} for 1965 and 3.9×10^{-6} for 1978. Presumably, risks from DDT in the diet would be even less today than the 1978 estimate of risk, since DDT residues have undoubtedly dissipated since 1978.

8. DISCUSSION

The major concern of this report is the estimation of the carcinogenicity of dicofol. In 1978 a 2-year bioassay by the NCI on dicofol was found to be negative for carcinogenicity in both sexes of Osborne-Mendel rats, and also negative in female B6C3F1 mice. The response in male B6C3F1 mice, however, was positive, consisting of hepatocellular carcinomas. Dicofol is therefore judged to be a possible ($p > 0$) human carcinogen. Normally, on this limited basis, dicofol would be judged as belonging in EPA's Group C, but because of the large data base on DDT (EPA Group B2), the classification of dicofol is raised from Group C to the range of C to B2 due to the close similarities of the chemical structure of difocol to that of DDT and similarity in cancer potency estimates. To encompass the eventuality that difofol is, in fact, a human carcinogen, the CAG estimates the cancer potency of dicofol, on the basis of the hepatocellular carcinoma response in male B6C3F1 mice, to be $q_1^* = 0.44\ (mg/kg/day)^{-1}$.

Much more information has been obtained as to the carcinogenicity of DDT and DDE. In eight of nine studies using dietary DDT, mice showed benign and malignant liver tumors. In two multigeneration biotests, lung carcinomas and leukemias were observed (but not liver tumors). In two other multigeneration studies in mice, liver tumors observed (mostly benign liver tumors, sometimes referred to as hepatomas in the literature) did not increase with the successive generations. This rather flat response with passing generations tends to allay concerns about the cancerous effects of DDT being vertically transmitted. Clearly, however, tests in mice have been positive for DDT carcinogenicity.

Rats, in some contrast to mice, showed a limited carcinogenic response to DDT, with positive results only above a rather high dose of 25 mg/kg/day, only with benign liver tumors, and with the total number of rats with tumors invari-

ant among dosed groups and controls. Hamsters fed DDT did not respond with excess tumors but did show a weak response to DDE. Fish developed benign liver tumors with limited exposure. Dogs and monkeys did not respond with tumors, although these studies were not conducted for long enough periods, or with enough animals, to firmly establish negative carcinogenicity. All of these biotest results, taken together, were not considered to be sufficient evidence of carcinogenicity according to the IARC's or EPA's proposed classification scheme. The results for carcinogenicity represented more than one positive test species (mice), but not as much as two positive test species.

It seems clear that the rat is more refractory to DDT in the diet than the mouse. This conclusion is based on the following factors: (1) rats formed only benign tumors, (2) excess tumors were observed only in the liver and not in the lung, (3) excess tumors were observed only above a certain dose ($\geq$ 25 mg/kg/day), and (4) only the tumor pattern was changed in those experiments in which increased liver tumors were observed, since the number of tumor-bearing animals was the same for control and treated groups. On the other hand, (1) mice formed benign and malignant tumors, (2) tumors were observed in more than one organ (liver and lung tumors, and sometimes leukemia), (3) tumors were observed at all doses from 0.15 to 37.5 mg/kg/day (although the lower doses in the Shabad et al. (1973) and the Tarjan and Kemeny (1969) studies were multi-generation exposures), and (4) increased numbers of tumor-bearing animals and tumor loads (multiplicity), were observed, as well as increased organ-specific tumors (i.e., liver and lung). These differences indicate species variability in response to DDT administered in the diet.

It should be noted that the propensity of B6C3F1 mice to respond to chlorohydrocarbon compounds (such as DDT) with liver tumors, which are usually benign adenomas, has been reviewed and cited as a potential problem in inter-

preting the oncogenic risk of these compounds to humans (Doull et al., 1983, p. 29). Since the metabolism in the mouse is similar to the metabolism in humans (WHO, 1979), dicofol should be considered a potential cancer problem. To date, however, there have been no epidemiologic studies on dicofol in humans. Furthermore, carcinomas and not adenomas were identified in male mouse livers, thereby making consideration of the cancer potential of dicofol more compelling and necessary.

The negative DDT data in the hamster indicate that, although present in hamster tissues, DDT is not carcinogenic even at high doses. On the other hand, DDE was active in the hamster, but only mildly so; no change was observed in total tumor-bearing animals among controls and dosed groups, and only neoplastic nodules (not malignant tumors) were produced. The failure of such high doses of DDT to cause tumors, while DDE does cause some tumors at these doses, suggests that perhaps DDT is a procarcinogen and that DDE is a proximate carcinogen in the hamster. An explanation of the inactivity of DDT in the hamster has been given by Gold and Brunk (1983). They found that DDT is stored in animals' bodies, but is poorly converted to DDE. It is not likely that this is the case in humans, since both DDT and DDE are found to occur in human body fat throughout the world wherever DDT has been used; moreover, it has been found that man, unlike the hamster, can convert DDT to DDE (WHO, 1979). However, the CAG concludes that these results could be unique to the hamster, since similar responses have not been demonstrated in other species.

The degree of malignancy produced by DDT was somewhat variable in biotests that were positive for carcinogenicity. Rats produced only neoplastic liver nodules and benign liver tumors best designated as hepatocellular adenomas. Mice produced nodules and carcinomas, but in no case was there DDT-induced dissemination of cells leading to metastasis. In a study by Tomatis et al.

(1974a), limited dosing followed by sequential sacrifices showed that (1) the mouse hepatomas grew in size and number with continued time of DDT dosing, (2) the hepatomas maintained growth even after dietary DDT was removed, and (3) mice with hepatomas died somewhat earlier. The latter observation, however, was generally not substantiated by most of the other studies reviewed in this document. In one study, the benign liver tumors continued to grow after being transplanted into syngenetic mice. All of the above observations suggest a malignant character of the DDT-induced liver tumors, but not enough to definitely cause the death of tumor-bearing animals, or to cause the spreading of the cancer to other organ sites.

Additional support for the carcinogenicity of DDT was gained from positive genotoxicity results. The types of tests that were positive, i.e., increased point mutations, chromosome aberrations, increased frequency of sister chromatid exchange, and direct interaction with DNA, suggest that these positive results could portend genotoxic effects in man. It has been theoretically suggested that rearrangement of oncogene segments in DNA from transcriptionally inactive to active regions can lead to tumor formation and progression (Klein and Klein, 1984).

Furthermore, recent studies on the classic tumor promoters TPA (12-O-tetra decanoyl-phorbol-13-acetate) and teleocidin in CH3 $10T_{1/2}$ fibroblast cells indicate that oncogene-induced transformation is enhanced irreversibly at the time of transfection by these tumor promoters (Hsiao, 1984). The CAG views these mechanisms as possible for DDT, although DDT has been historically thought by some to act (when positive for carcinogenicity) as a tumor promoter only by acting via epigenetic mechanisms. The positive genotoxicity suggests the potential for oncogene activation by DDT, and thus would indicate a tumor-initiation capacity for DDT. The initiation capacity, plus the well-recognized

promotion capacity (discussed in Section 5.1.), constitute complete carcinogenic activity for DDT. Complete carcinogenic activity is, in fact, observed in the mouse even at low dietary doses of DDT, which could indicate that the mouse studies reflect the true carcinogenic potential of DDT. Such a conclusion would be in contradistinction to the hypothesis that chlorohydrocarbons may be unusually sensitive in the mouse (Doull et al., 1983).

Additional support for the carcinogenicity of DDT is given by the ability of DDT to promote the tumor-initiation activity of diethylnitrosamine (liver), trans-4-acetylaminostilbene (mammary gland), 2-acetylaminofluorene (liver), and 2-acetamidophenanthrene (liver). Such a capacity to interface with different known initiators enhances the idea that DDT has intrinsic promotion capacity. DDT has also been compared to phenobarbital and was found to be similar to this well-recognized liver tumor promoter; such a similarity again adds to the idea that DDT has tumor promotion characteristics. Both DDT and phenobarbital are thought to incorporate into liver cell plasma membranes, thereby interrupting cellular communication, disassociating cell-field integrity, and evolving a progressively unregulatable neoplasm. Tetradecanoylphorbol acetate, a well-known tumor promoter in mouse skin, is thought to generate free radicals at the plasma membrane, thereafter leading to tumor-promotion sequelae similar to those of DDT and phenobarbital.

The genotoxicity and tumor-promotion results offer enough additional evidence for the carcinogenicity of DDT to raise the estimation for carcinogenicity to the equivalent of two positive animal species. Since inadequate human data exist for DDT, the IARC classification for DDT is Group 2B (EPA's Group B2).

The CAG has reviewed the carcinogenicity of two other compounds that bear structural similarity to dicofol, DDT, DDE, and DDD. The compounds are chlorobenzilate (CAG, 1978) and Perthane™ (CAG, 1977). Both compounds (structures

given in Table 1) induced liver tumors similar to those induced by dicofol, DDT, DDE, and DDD, and dicofol, but at higher feed concentrations.

Some light is shed on the potential human cancer potency of dicofol, DDT, DDE, and DDD when the q_1^* values for each of these chemicals are summarized by taking the geometric mean of each of the studies within each chemical group. Surprisingly, the q_1^* values are quite close in magnitude:

	Dicofol	DDT	DDE	DDD
q_1^* $(mg/kg/day)^{-1}$ =	0.44	0.34	0.34	0.24

The comparability of these results in terms of cancer potency is remarkable, given the diverse bioassay conditions under which the positive data were obtained and the assumptions of the mathematical unit risk estimation process. The CAG views these similarities in q_1^* values as having the following possible meanings:

1. The compounds are similar in intrinsic carcinogenic activity (at least in Rodentia); or
2. The compounds share a common metabolite or a common impurity which is the cause of the carcinogenic process.*

At the present time it is not possible to distinguish between these alternatives.

The CAG recommends that technical grades of dicofol, DDT, DDE, and DDD all be considered potential human carcinogens, and that an aggregate estimate of the upper confidence limit on the cancer potency of 0.34 $(mg/kg/day)^{-1}$ be used in the risk management of these compounds. The potency index (q_1^* x molecular weight) for DDT is $1.20 \times 10^{+2}$ $(mmol/kg/day)^{-1}$, which places DDT, the other DDT

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*Figures 1 and 2 present a theoretical scheme for carcinogenic activity that shows the interrelation of these compounds (Figure 1), and a putative reactive intermediate in DDE ring oxidation (Figure 2), which could be the compound for carcinogenesis.

analogues discussed in this document, and technical-grade dicofol in the third quartile of potency for compounds reviewed by the CAG.

It should be understood that any incremental risks incurred from dicofol use should be compared, during the risk-management phase, to risks extant in the United States from the presence of DDT, DDE, and DDD in the soil and the biotic communities related to those use areas. DDT, DDE, and DDD have been found throughout the world in the food chain up to and including humans (WHO, 1979). The persistence of DDT residues in soil is likely to be quite long, with a half-life estimated to be approximately 12 years. Humans in the United States are exposed to these substances, even 12 years after the ban on DDT, from conceptus until death. DDT and structurally related compounds have been found in fetuses, neonates (mother's milk contains DDT), and adults. This persistence is mainly due to the slow breakdown of these compounds in the environment, their very high lipid solubility in body fat, and the very slow in vivo breakdown of DDT and DDE. The half-life of DDE in human body fat may be seven decades, while that of DDT may be two decades (WHO, 1979). The pervasiveness of DDT that was placed in the environment years ago, therefore, could affect any considerations of the present use of dicofol.

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